APR 13 1938
MEDICAL LIBRARY

Volume XI Number 2

Physiological Zoölogy

APRIL, 1938

Annotated Bibliography of the Scientific Publications of Professor Charles Manning Child - - - - - - - - - - *Libbie H. Hyman and C. D. Van Cleave* 105

The Fragmentation of *Nais paraguayensis* - - - - - - *Libbie H. Hyman* 126

The Effect of Planarian Extracts and Exudates upon Head Regeneration in *Euplanaria dorotocephala* - - - - - - - - - - - *J. William Buchanan* 144

The Microscopic Anatomy of Twins and Double Monsters of *Fundulus heteroclitus* *Marie A. Hinrichs* 155

The Influence of Thyroxin on Eye Formation in *Phagocata gracilis* (Haldeman) *Bernal R. Weimer, Charles W. Phillips, and Dagney M. Andersen* 158

The Effect of Dead Optic Vesicle upon Explants of Prospective Ectoderm *C. D. Van Cleave* 168

Oxygen as a Controlling Factor in the Regeneration of Tubularia - *L. G. Barth* 179

Differential Gradient Functions in the Feather Germ - - - *Richard M. Fraps* 187

Single and Combined Effects of Cyanide and Methylene Blue on Reconstitution in *Euplanaria dorotocephala* - - - - - - - - - - - - *Olin Rulon* 202

Studies on Heteroplastic Transplantation in Triclads. I. Cephalic Grafts between *Euplanaria dorotocephala* and *E. tigrina* - - - - - - *James A. Miller* 214

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

WARDER C. ALLEE
Managing Editor

EDITORIAL BOARD

J. H. BODINE, *University of Iowa*
C. M. CHILD, *Stanford University*
M. F. GUYER, *University of Wisconsin*
L. V. HEILBRUNN, *University of Pennsylvania*
E. E. JUST, *Howard University*
F. R. LILLIE, *University of Chicago*
R. S. LILLIE, *University of Chicago*
W. W. SWINGLE, *Princeton University*
B. H. WILLIER, *University of Rochester*
C. V. TAYLOR, *Stanford University*

SPECIAL EDITORIAL COMMITTEE FOR THIS ISSUE

LIBBIE H. HYMAN, *American Museum of Natural History*
JAMES WILLIAM BUCHANAN, *Northwestern University*
LESTER GEORGE BARTH, *Columbia University*

Physiological Zoölogy is published quarterly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. The subscription price is $7.50 a year; the price of single copies is $2.25. Orders for service of less than a half-year will be charged at the single-copy rate. Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Puerto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, Canary Islands, El Salvador, Argentina, Bolivia, Brazil, Colombia, Chile, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Peru, Hayti, Uruguay, Paraguay, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Balearic Islands, Spain, and Venezuela. Postage is charged extra as follows: for Canada and Newfoundland, 15 cents on annual subscriptions (total $7.65), on single copies 4 cents (total $2.29); for all other countries in the Postal Union, 40 cents on annual subscriptions (total $7.90), on single copies 10 cents (total $2.35). Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized agents:

For the British Empire, except North America, India, and Australasia: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Prices of yearly subscriptions and of single copies may be had on application.

For Japan: The Maruzen Company, Ltd., Tokyo.

For China: The Commercial Press, Ltd., 211 Honan Road, Shanghai. Yearly subscriptions, $7.50; single copies, $2.25, or their equivalents in Chinese money. Postage extra on yearly subscriptions 40 cents, on single copies 10 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editors and manuscripts should be addressed to W. C. Allee, Managing Editor of PHYSIOLOGICAL ZOÖLOGY, The University of Chicago, Chicago, Ill.

The articles in this journal are indexed in the *International Index to Periodicals*, New York, N.Y.

Applications for permission to quote from this journal should be addressed to The University of Chicago Press, and will be freely granted.

Entered as second-class matter October 27, 1928, at the post-office at Chicago, Illinois, under the Act of March 3, 1879.

[PRINTED IN U.S.A.]

PHYSIOLOGICAL ZOÖLOGY

A Quarterly Journal of Zoölogical Research

WARDER C. ALLEE
Managing Editor

EDITORIAL BOARD

J. H. BODINE, *University of Iowa*
C. M. CHILD, *Stanford University*
M. F. GUYER, *University of Wisconsin*
L. V. HEILBRUNN, *University of Pennsylvania*
E. E. JUST, *Howard University*
F. R. LILLIE, *University of Chicago*
R. S. LILLIE, *University of Chicago*
W. W. SWINGLE, *Princeton University*
B. H. WILLIER, *University of Rochester*
C. V. TAYLOR, *Stanford University*

SPECIAL EDITORIAL COMMITTEE FOR THIS ISSUE

LIBBIE H. HYMAN, *American Museum of Natural History*
JAMES WILLIAM BUCHANAN, *Northwestern University*
LESTER GEORGE BARTH, *Columbia University*

Physiological Zoölogy is published quarterly by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. The subscription price is $7.50 a year; the price of single copies is $2.25. Orders for service of less than a half-year will be charged at the single-copy rate. Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Puerto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, Canary Islands, El Salvador, Argentina, Bolivia, Brazil, Colombia, Chile, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Peru, Hayti, Uruguay, Paraguay, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Balearic Islands, Spain, and Venezuela. Postage is charged extra as follows: for Canada and Newfoundland, 15 cents on annual subscriptions (total $7.65), on single copies 4 cents (total $2.29); for all other countries in the Postal Union, 40 cents on annual subscriptions (total $7.90), on single copies 10 cents (total $2.35). Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized agents:

For the British Empire, except North America, India, and Australasia: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Prices of yearly subscriptions and of single copies may be had on application.

For Japan: The Maruzen Company, Ltd., Tokyo.

For China: The Commercial Press, Ltd., 211 Honan Road, Shanghai. Yearly subscriptions, $7.50; single copies, $2.25, or their equivalents in Chinese money. Postage extra on yearly subscriptions 40 cents, on single copies 10 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

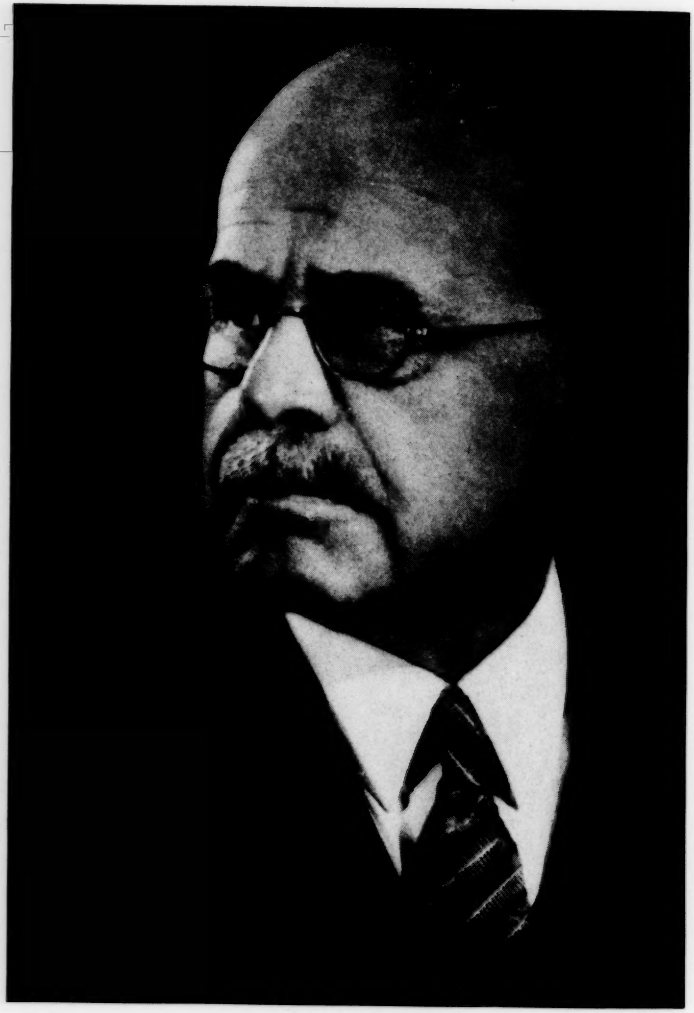
Communications for the editors and manuscripts should be addressed to W. C. Allee, Managing Editor of PHYSIOLOGICAL ZOÖLOGY, The University of Chicago, Chicago, Ill.

The articles in this journal are indexed in the *International Index to Periodicals*, New York, N.Y.

Applications for permission to quote from this journal should be addressed to The University of Chicago Press, and will be freely granted.

Entered as second-class matter October 27, 1928, at the post-office at Chicago, Illinois, under the Act of March 3, 1879.

[PRINTED IN U.S.A.]



C. M. Child.

UNIV. OF MICH.

CHARLES MANNING CHILD

Professor Emeritus of Zoölogy
The University of Chicago

This number of *Physiological Zoölogy* is made up of papers by former students of Professor Child who wrote their Doctor's theses under his supervision. Dr. Libbie H. Hyman, J. William Buchanan, and Lester G. Barth have assisted in the editorial work.

CHARLES MANNING CHILD—VITA

1869 February 2, born Ypsilanti, Michigan, of New England parentage
1890 Ph.B., Wesleyan University, Middletown, Connécticut (Chi Psi Fraternity)
1890–92 Graduate assistant in biology, *ibid.*
1892 S.M., *ibid.*
1894 Ph.D., University of Leipzig, Germany
1894 Naples Zoölogical Station
1894 Assistant in zoölogy, University of Chicago
1895 Associate, *ibid.*
1897 Instructor, *ibid.*
1895–97 Instructor in embryology, Marine Biological Laboratory
1898 Assistant professor of zoölogy, University of Chicago
1899 Married Lydia Van Meter of Baltimore, Maryland
1902 Naples Zoölogical Station
1909 Associate professor of zoölogy, University of Chicago
1911 *Die physiologische Isolation von Teilen des Organismus* (Leipzig)
1915 *Senescence and Rejuvenescence* (Chicago)
1915 *Individuality in Organisms* (Chicago)
1916 Professor of zoölogy, University of Chicago
1919 President, American Society of Zoölogists
1921 *The Origin and Development of the Nervous System from a Physiological Point of View* (Chicago)
1924 *Physiological Foundations of Behavior* (New York)
1928 Joined with W. C. Allee to found the journal, *Physiological Zoölogy*, and became first managing editor
1928 Honorary D.Sci., Wesleyan University, Connecticut
1929 Vice-president, Section F, American Association for the Advancement of Science
1930 Visiting professor, Duke University, from February to June
1930–31 Rockefeller Foundation Visiting Professor, Tohoku Imperial University, Sendai, Japan
1932 Chairman, Department of Zoölogy, University of Chicago
1933 Elected to the National Academy of Science
1934 Professor emeritus of zoölogy, *ibid.*
1937 Moved to Palo Alto, California; present address: Jordan Hall, Stanford University, California
1895—— Research worker at the marine biological laboratories at Woods Hole, Friday Harbor, La Jolla, Pacific Grove, and Asamushi (Japan)

Physiological Zoölogy

Vol. XI APRIL 1938 *No. 2*

ANNOTATED BIBLIOGRAPHY OF THE SCIENTIFIC PUBLICATIONS OF PROFESSOR CHARLES MANNING CHILD

LIBBIE H. HYMAN
American Museum of Natural History, New York City
AND
C. D. VAN CLEAVE
Department of Anatomy, University of Pennsylvania

1892*a*. Statistics of "unconscious cerebration." Amer. Jour. Psychol., **5**:249–59.

1892*b*. The functions of the nervous system of the Myriapoda. Amer. Nat., **26**:1051–55.

In the absence of head or cerebral ganglia, the trunk will, when stimulated, execute normal movements, but weaker and less exact. The effects of partial destruction of the cerebral ganglia are proportional to quantity destroyed; circus movements result when half is destroyed. No difference was found in trunk behavior with respect to level.

1894*a*. Beiträge zur Kenntnis der antennalen Sinnesorgane der Insekten. Zool. Anz., **17**:35–38.

1894*b*. Contributions to the knowledge of the antennary sense-organs of insects. Ann. and Mag. of Nat. Hist., **13**:372–74.

A translation of 1894*a*. Surveys the occurrence of the antennary sense organ in the orders of insects.

1894*c*. Ein bisher wenig beachtetes antennales Sinnesorgan der Insekten mit besonderer Berücksichtigung der Culiciden und Chironomiden. Zeitschr. f. wiss. Zool., **58**:475–528.

This, Professor Child's thesis at the University of Leipzig, has become a standard reference in entomological works. A detailed account of the histology and innervation of an antennary sense-organ, especially in the lower Diptera; perception of vibrations is suggested as the function.

1896. Translation of E. HAASE: Researches on mimicry on the basis of a natural classification of the Papilionidae. Part II. Stuttgart and London. Pp. 154; 8 plates.

1897*a*. Centrosome and sphere in the ovarian stroma of mammals. Abstract, Zoölogy Club, University of Chicago, meeting of January 6, 1897. Science, N.S., **5**:231–32.

1897*d*. Centrosome and sphere in cells of the ovarian stroma of mammals. A preliminary communication. Zoöl. Bull., **1**:87–94.

Stroma cells of the pregnant ovary of rabbit and dog contain spheres and centrioles, although they do not divide.

1897*b*. I. Cleavage of the egg of *Arenicola*. II. The oblique cleavage and its relation to the mosaic theory. Abstract, Zoölogical Club, University of Chicago. Science, N.S., **5**:629–31.

1897*c*. A preliminary account of the cleavage of *Arenicola cristata*, with remarks on the mosaic theory. Zoöl. Bull., **1**:71–85.

1898*a*. Some features of the oögenesis of *Sternaspis*. Science, N.S., **7**:104–5.

1898*b*. The maturation and fertilization of the egg of *Arenicola marina*. Trans. N.Y. Acad. Sci., **16**:387–94.

1898*c*. The axes of the annelid egg. Science, N.S., **8**:140.

1900*c*. The significance of the spiral type of cleavage and its relation to the process of differentiation. Biol. Lectures, Marine Biol. Lab. Woods Hole, for 1899, pp. 231–65.

1900*d*. The early development of *Arenicola* and *Sternaspis*. Arch. f. Entw'mech., **9**:587–723.

Final extensive paper to which the preceding six are preliminary; a classic on cell lineage. Spiral cleavage is interpreted as a form of cleavage offering the least physical obstacle to intercommunication of the components. Bilateral cleavage is later superimposed on the spiral type and leads directly to the formation of the larva; hence called "morphogenetic cleavage." In annelids and mollusks morphogenetic cleavage is increased at the expense of spiral cleavage. The cleavage pattern of different species is identical only as long as the spiral type obtains. The differentiation of larval organs is related to their functional necessity, and hence may occur at different times in the development of different species. Bilateral cleavage is interpreted as accomplishing a symmetrical distribution of materials to the larva. The mosaic theory is rejected on the grounds of the failure of exact cell homology in many cases, the differences in size and structure of blastomeres having the same fate, and the ability of portions of determinate embryos to regulate to at least some extent. A strictly cellular theory of development must always be inadequate; the explanation of development must be sought in the organization of the egg and of each stage as a whole. Hertwig's view that the fate of a cell is a function of its position is accepted.

1900*a*. The egg of the *Stichostemma*. Science, N.S., **11**:249–50.

1901*c*. The habits and natural history of *Stichostemma*. Amer. Nat., **35**:975–1006.

The external features, occurrence, movements, reactions, food, and enemies of the fresh-water nemertean are described. The habit of forming a slime coat may have protective value. Self-fertilization is probably the rule. Each ovary is a sac with a syncytial wall; this wall gradually bulges into the cavity as a single egg, which finally constricts off and flows through the small pore to the exterior.

1900*b*. A specimen of *Nais* with a bifurcated prostomium. Anat. Anz., **17**:311–12.

1900*e*. Abnormalities in cestodes. Abstract, Zoölogical Club, University of Chicago. Science, N.S., **12**:28–29.

1900*f*. Abnormalities in the cestode *Moniezia expansa*. I. Imperfect and partial proglottids. Biol. Bull., **1**:215–50.

1900*g*. Abnormalities in the cestode *Moniezia expansa*. II. 1. Spiral abnormalities. 2. Other abnormalities. Biol. Bull., **1**:261–90.

1902*b*. Abnormalities in the cestode *Moniezia expansa*. III. Biol. Bull., **3**:95–114, 143–60.

The abnormalities consist chiefly of imperfect separation of proglottids. Study of the development of the proglottids and their organs showed that the external abnormalities are but a surface expression of internal developmental anomalies.

1901*a*. Regulation in *Stenostomum*. Abstract, Zoölogical Club, University of Chicago. Science, N.S., **14**:28–29.

1901*b*. Fission and regulation in *Stenostomum leucops*. Abstract, Meeting of American Morphological Society, Baltimore, December, 1900. Biol. Bull., **2**:329–31.

1902*a*. An experimental study of regulation in *Stenostoma*. Science, N.S., **15**:527–28.

1902*c*. Studies on regulation. I. Fission and regulation in *Stenostoma*. Part I. The structure and movements of *Stenostoma* and the process of fission. Part II. The theory of mechanical regulation. Arch. f. Entw'mech., **15**:187–237.

1902*d*. Studies on regulation. I. Fission and regulation in *Stenostoma*. Part III. The regulation of zooids and groups of zooids. Arch. f. Entw'mech., **15**:355–420.

1903*b*. Studies on regulation. II. Experimental control of form-regulation in zooids and pieces of *Stenostoma*. Arch. f. Entw'mech., **15**:603–37.

1903*e*. Studies on regulation. III. Regulative destruction of zooids and parts of zooids of *Stenostoma*. Arch. f. Entw'mech., **17**:1–40.

These papers on *Stenostomum* are the first of a long series of researches on the role of mechanical factors, especially tensions resulting from locomotion and attachment, in form-regulation. *Stenostomum* attaches by the tail and ventral surface; such tensions induce a conoidal form. Regulation can be controlled by preventing attachment through keeping the pieces moving; this inhibits or delays the formation of a tail. Typical increase in length, decrease in breadth, of regenerating pieces result from tail attachment and are greatest in pieces with heads, since these attach. Pieces without heads do not attach, and change shape slowly. *Stenostomum* has no "normal" form; its form is the result of its activities; morphallaxis is brought about by differences in tensions. In pieces containing parts of more than one zooid, the oldest zooid absorbs the younger zooids or portions thereof anterior to it.

1903*a*. With A. N. Young. Regeneration of the appendages in nymphs of the Agrionidae. Arch. f. Entw'mech., **15**:543–602.

Emphasis is placed on the correlation between the regeneration of muscles and tendons and the regeneration of legs and formation of joints. Normal segmentation of the leg into joints depends on regeneration of normal muscle arrangements; the tarsal claws do not become articulated unless tendons reach them.

1903*f*. Form-regulation in Coelentera and Turbellaria. Smithsonian Misc. Coll. **45**:134–43.

Summary of work on the regeneration of *Leptoplana*, *Cestoplana*, *Cerianthus*, and *Tubularia*.

1903*c*. Form-regulation in *Cerianthus*. I. The typical course of regeneration. Biol. Bull., **5**:239–60.

1903*d*. Form-regulation in *Cerianthus*. II. The effect of position, size, and other factors upon regeneration. Biol. Bull., **5**:304–19. II. Continued. Biol. Bull., **6**:1–11.

1904*a*. Form-regulation in *Cerianthus*. III. The initiation of regeneration. Biol. Bull., **6**:55–74.

1904*b*. Form-regulation in *Cerianthus*. IV. The role of water-pressure in regeneration. Biol. Bull., **6**:266–86.

1904*d*. Form-regulation in *Cerianthus*. V. The role of water-pressure in regeneration: further experiments. Biol. Bull., **7**:127–53.

1904*e* Form regulation in *Cerianthus*. VI. Certain special cases of regulation and their relation to internal pressure. Biol. Bull., **7**:193–214.

1904*f*. Form regulation in *Cerianthus*. VII. Tentacle-reduction and other experiments. Biol. Bull., **7**:263–79.

1905*a*. Form regulation in *Cerianthus*. VIII. Supplementary partial discs and heteromorphic tentacles. Biol. Bull., **8**:93–122.

1905*b*. Form regulation in *Cerianthus*. IX. Regulation, form and proportion. Biol. Bull. **8**:271–89.

1908*b*. Form regulation in *Cerianthus aestuarii*. Biol. Bull., **15**:27–53.

Cerianthus pieces collapse; the ends close; and the piece distends through water intake. Such distension is a necessary condition for normal regeneration. If prevented by keeping the aboral end open, regeneration is delayed or inhibited, and regenerated tentacles are short and stumpy. The direct factors are probably water currents striking against the anterior end of the piece. Regeneration is greatest in rate and amount orally and decreases basally. In oblique pieces the most anterior angle regenerates first. Regeneration is not proportional to the size of the piece but is relatively greater the smaller the piece. The change of form of pieces in the direction of normal elongated shape occurs chiefly after the regeneration of the oral end.

1904*c*. Studies on regulation IV. Some experimental modifications of form-regulation in *Leptoplana*. Jour Exper. Zoöl., **1**:95–133.

1904*g*. Studies on regulation. V. The relation between the central nervous system and regeneration in *Leptoplana:* posterior regeneration. Jour. Exper. Zoöl., **1**:463–512.

1904*i*. Studies on regulation. VI. The relation between the central nervous system and regeneration in *Leptoplana:* anterior and lateral regeneration. Jour. Exper. Zoöl., **1**:513–57.

1905*c*. Studies on regulation. VII. Further experiments on form-regulation in *Leptoplana*. Jour. Exper. Zoöl., **2**:253–85.

Further pursuit of the idea of the importance of pressure and tension in regeneration. In curved pieces of the polyclad *Leptoplana* the regenerated tail curves in the direction of the principal tension. Regions develop in a typical form because they function in a characteristic manner. The regenerated tail is shorter and less pointed the less active the piece. The amount and completeness of anterior and lateral regeneration are also correlated with the characteristic motor activity. Changes in proportions are the result of tensions during locomotion.

1905*d*. Studies on regulation. VIII. Functional regulation and regeneration in *Cestoplana*. Arch. f. Entw'mech., **19**:261–94.

1905*f*. Studies on regulation. IX. The positions and proportions of parts during regulation in *Cestoplana* in the presence of the cephalic ganglia. Arch. f. Entw'mech., **20**:48–75.

1905*g*. Studies on regulation. X. The positions and proportions of parts during regulation in *Cestoplana* in the absence of the cephalic ganglia. Arch f. Entw'mech., **20**:157–86.

1907*o*. Studies on regulation. XI. Functional regulation in the intestine of *Cestoplana*. Jour. Exper. Zoöl., **4**:357–98.

1910*e*. The central nervous system as a factor in the regeneration of polyclad Turbellaria. Biol. Bull., **16**:277–96.

Pieces with ganglia elongate and become tapering, owing to locomotor activity. Pieces without ganglia regenerate but little; they undergo redifferentiation of the digestive branches in relation to mechanical conditions. In headless pieces the pharynx is more anterior the more posterior the level; pieces behind the old pharynx do not form a new pharynx. In starvation the intestine degenerates from periphery to center and from the ends toward the pharynx. In regeneration, branches of the intestine degenerate and new ones arise, corresponding in arrangement to the appropriate body region. In headless pieces without pharynx the intestine regenerates. Degeneration of old intestinal branches and re-formation of new ones are assigned to mechanical conditions.

1905*e*. Regeneration in nudibranchs. Science, N.S., **21**:851.

1906*a*. Contributions toward a theory of regulation. I. The significance of the different methods of regulation in Turbellaria. Arch. f. Entw'mech., **20**:380–426.

1906*e*. The relation between functional regulation and form-regulation. Jour. Exper. Zoöl., **3**:559–82.

1907*d*. The localization of different methods of form-regulation in *Polychoerus caudatus* Arch. f. Entw'mech., **23**:227–48.

These four papers continue the thesis that regulation results from the regenerating part acting functionally like the part removed. In different forms and different levels of the same form, redifferentiation (morphallaxis) and regeneration proper occur to different degrees. The more the region where a part was removed is able to substitute functionally for the part removed, the greater the role of redifferentiation of old tissue in the regulation; and regeneration occurs when functional substitution is limited to the cut surface and immediately adjacent tissue. Regulation is defined as a return or approach to a physiological or functional equilibrium after such equilibrium has been disturbed.

1906*c*. Some considerations regarding so-called "formative substances." Biol. Bull., **11**:165–81.

Proponents of formative substance theories have failed to understand that, since organic structure is essentially a space arrangement of materials, formative substances would have to act by determining such an arrangement. A gradation of head-forming or tail-forming substances would have in itself to be explained; further head and tail are highly complex parts. Formative substance theories err not only in these points but in failing to recognize that all processes are part of the functional activity of the organism.

1907*j*. Some corrections and criticisms. Arch. f. Entw'mech., **24**:131–46.

1907*f*. An analysis of form-regulation in *Tubularia*. I. Stolon-formation and polarity. Arch. f. Entw'mech., **23**:396–414.

1907*g*. An analysis of form-regulation in *Tubularia*. II. Differences in proportion in the primordia. Arch. f. Entw'mech., **23**:415–44.

1907*h*. An analysis of form-regulation in *Tubularia*. III. Regional and polar differences

in the relation between primordium and hydranth. Arch. f. Entw'mech., **23**: 445–56.

1907*i*. An analysis of form-regulation in *Tubularia*. IV. Regional and polar differences in the time of hydranth-formation as a special case of regulation in a complex system. Arch. f. Entw'mech., **24**:1–28.

1907*n*. An analysis of form-regulation in *Tubularia*. V. Regulation in short pieces. Arch. f. Entw'mech., **24**:285–316.

1907*o*. An analysis of form-regulation in *Tubularia*. VI. The significance of certain modifications of regulation: polarity and form-regulation in general. Arch. f. Entw'mech., **24**:317–49.

1909*b*. The regulation of mutilated primordia in *Tubularia*. Arch. f. Entw'mech., **27**: 106–13.

In *Tubularia* pieces the tendency to form stolons aborally is greater the more proximal the piece. The proportions of the parts of the primordium of the hydranth vary with level of piece. Hydranths form earlier on distal than on proximal pieces, and on longer than on shorter pieces; aboral hydranths arise later than oral on a piece. The shorter the piece, the longer the hydranth in proportion, until very short pieces become all hydranth with no stalk. Very short pieces produce simple or double complete or incomplete hydranths. The formation of an aboral hydranth is a case of asexual reproduction. If the physiological difference between the two ends of a piece is sufficiently great, a single structure of reduced size results; if the difference is slight, bipolar structures result. The polarity of *Tubularia* is not fixed but reacts to, and is alterable by conditions.

1906*b*. The relation between regulation and fission in *Planaria*. Biol. Bull., **11**:113–23.

1908*a*. Physiological basis of restitution of lost parts. Jour. Exper. Zoöl., **5**:485–502. Autoreferat in Arch. f. Entw'mech., **26**:501.

1909*b*. The regulatory change of shape in *Planaria dorotocephala*. Biol. Bull., **16**:277–96.

Application of the theory of the role of function in regulation to *Planaria;* a part can be replaced only when some remaining part is physiologically sufficiently similar to it to perform its functions at least qualitatively. The position of the pharynx in regenerating pieces depends on how much such pieces react like heads or tails, respectively. In dilute anesthetics, which prevent locomotion but permit regeneration, the normal change of shape of pieces does not occur; such change of shape is dependent on locomotion. The presence of a posterior zooid in *Planaria* is indicated for the first time.

1908*c*. Driesch's harmonic equipotential systems in form-regulation. Biol. Centralbl., **28**:577–88, 609–23.

A postulate of Driesch's system is retention of proportionality with every reduction. Proportionality is not retained with reduction of size of *Tubularia* pieces but varies with level and size. The postulate that in organisms, in contrast with machines, each part can do everything is also not in accord with the facts of regeneration.

1908*d*. Regulation of *Harenactis attenuata* in altered environment. Biol. Bull., **16**:1–17.

1909*a*. Experimental control of certain regulatory processes in *Harenactis attenuata*. Biol. Bull., **16**:47–53.

1909*c*. Factors of form regulation in *Harenactis attenuata*. I. Wound reaction and restitution in general and the regional factors in oral restitution. Jour. Exper. Zoöl., **6**:471–506. Autoreferat in Arch. f. Entw'mech., **28**:440.

1909*d*. Factors of form regulation in *Harenactis attenuata*. II. Aboral restitution, heteromorphosis and polarity. Jour. Exper. Zoöl., **7**:65–96.

1909*e*. Factors of form regulation in *Harenactis attenuata*. III. Regulation in "rings." Jour. Exper. Zoöl., **7**:353–95. Autoreferat in Arch. f. Entw'mech., **29**:198–99.

1910*f*. Further experiments on adventitious reproduction and polarity in *Harenactis*. Biol. Bull., **20**:1–13.

On removal from its sand burrow the slender *Harenactis* slowly shortens to a typical anemone form with expanded oral and pedal disks; external conditions therefore determine the shape. The results of a cut into the side of the upper end depend on internal distension; without distension, a part degenerates. Level of piece determines rate of closure, amount of restitution, number of tentacles, etc. Pieces through the esophagus usually regenerate biaxial oral ends; below the esophagus no aboral head arises. The regeneration of the foot is greatest aborally and decreases distally. Regional differences are both qualitative and quantitative. Rings result from the union of the two cut surfaces into a doughnut shape if mesenteries and esophagus are removed. Such rings regenerate irregular tentacles or groups of tentacles along or near the line of union. The old polarity is lost, and tentacle groups represent adventitious new polarities.

1904*h*. Amitosis in *Moniezia*. Anat. Anz., **25**:545–58.

1906*d*. The development of germ cells from differentiated somatic cells in *Moniezia*. Anat. Anz., **29**:592–97.

1907*a*. Studies on the relation between amitosis and mitosis. I. Development of the ovaries and oögenesis in *Moniezia*. Biol. Bull., **12**:89–114.

1907*b*. Studies, etc. II. Development of the testis and spermatogenesis in *Moniezia*. Biol. Bull., **12**:175–90.

1907*c*. Studies, etc. II. Continued. Biol. Bull., **12**:191–224.

1907*e*. Amitosis as a factor in normal and regulatory growth. Anat. Anz., **30**:271–97.

1907*k*. Studies, etc. III. Maturation, fertilization, and cleavage in *Moniezia*. Biol. Bull., **13**:138–60.

1907*l*. Amitosis and mitosis in normal and regulatory growth. Proc. Seventh Intl. Zoöl. Congress, pp. 344–46.

1907*p*. Studies, etc. IV. Nuclear division in the somatic structures of the proglottids of *Moniezia*. V. General discussion and conclusions concerning amitosis and mitosis in *Moniezia*. Biol. Bull., **13**:165–84.

1910*a*. The occurrence of amitosis in *Moniezia*. Biol. Bull., **18**:109–19.

1911*e*. The method of cell division in *Moniezia*. Biol. Bull., **21**:280–96.

Amitosis was found to be the characteristic mode of division in rapidly growing regions, in the formation of the gonads and their ducts, nephridia, nerve cords, and interproglottidal glands. Testes may originate from muscle cells which divide amitotically to become a syncytial mass; the spermatogenic divisions are mitotic. The ovaries arise from parenchymal cells by amitotic divisions; maturation and early cleavages are mitotic, but later cleavages are amitotic. Amitosis is believed to be characteristic of rapidly growing and highly active regions, as in developing embryos and regenerating pieces of hydroids, planarians, etc.

1907*m*. Correlation in regulation. Proc. Seventh Intl. Zoöl. Congress, pp. 497–500.

1910*d*. Physiological isolation of parts and fission in *Planaria*. Arch. f. Entw'mech. (Festschrift für Roux, Vol. II), **30**:159–205.

1911*a*. Die physiologische Isolation von Teilen des Organismus als Auslösungsfaktor der

Bildung neuer Lebewesen und der Restitution. Vorträge und Aufsätze über Entwicklungsmechanik (ed. by W. Roux), **11**. Pp. 157.

The problem of the unity of the organism is the problem of correlation. The chief factor in correlation is antero-posterior dominance: each level dominates the region behind and is dominated by that in front. The domination of the anterior end over posterior regions declines with distance; hence each individual can reach only a limited size; beyond that size there occurs a physiological isolation, essentially similar to a physical isolation. Besides this distance factor, the length of the individual and the range of dominance of the head are dependent on the degree of activity of the dominant region, the efficiency of conduction, and the degree of receptivity of the posterior regions. The physiological isolation of posterior parts can be experimentally altered by way of these four factors. The results of the physiological isolation of parts depends on the capacities of these parts. If they are incapable of regeneration, physiological isolation results in atrophy, degeneration, absorption, or death of the parts in question. Physiological isolation probably accounts for the absorption or casting off of embryonic organs during metamorphosis; also for the self-differentiation of embryonic regions. If parts physiologically isolated are capable of regeneration, then asexual reproduction occurs. The distance factor is plainly evident in the formation of zooids in naids, planarians, and *Stenostomum*, in the budding of hydroids, etc. Physiological isolation may permit the transformation of parts under the action of external factors, as the change from vegetative to reproductive organs in plants. The localization of sex cells may be the result of physiological isolation. Fragmentation, adventitious budding, colony formation—all involve the principle of physiological isolation. Neoplasms may also be explained as an escape from physiological control.

In planarians increase in length results in the formation of zooids in the postpharyngeal region and consequent fission. Fission can be induced at a length much shorter than normal by starvation, decapitation, dilute anesthetics, rise of temperature, and can be delayed beyond the normal length by low temperature.

1910*c*. The regulatory processes in organisms. Jour. Morph., **22**:171–222.

The existence and continuity of organic individuality are dependent on physiological correlation. Correlative factors classify as mass, or mechanical; transportative; and transmissive, or dynamic. Continued existence is continued equilibration; regulation is the return to a state of dynamic equilibrium after the previous state has been disturbed, and embraces a wide range of phenomena, from a reflex act to regeneration. Life is a succession of equilibrations. The organism is compared to a river, whose structure is the result of past functioning but also influences present function. The form and shape of the river and its banks are the result of the activity of the stream; so, also, the organism is constructed by functioning, not for function. The regulatory processes classify as quantitative or compensatory and qualitative or transformative. In the regeneration of a piece there is a transformation of the whole piece, a reconstitution; it results from the attempt of the piece to function like the whole.

1911*b*. Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. I. The axial gradient in *Planaria dorotocephala* as a limiting factor in regulation. Jour. Exper. Zoöl., **10**:265–320.

1911*d*. Experimental control of morphogenesis in the regulation of *Planaria*. Biol. Bull. **20**:309–31.

1911*f*. Studies on the dynamics of morphogenesis, etc. II. Physiological dominance of anterior over posterior regions in the regulation of *Planaria dorotocephala*. Jour. Exper. Zoöl., **11**:187–220.

1911*g*. Studies on the dynamics of morphogenesis, etc. III. The formation of new zooids in *Planaria* and other forms. Jour. Exper. Zoöl., **11**:221–80.

1911*h*. With E. V. McKie. The central nervous system in teratophthalmic and teratomorphic forms of *Planaria dorotocephala*. Biol. Bull., **22**:39–60.

1912*b*. Antero-posterior dominance in *Planaria*. Abstract. Science, N.S., **35**:935–36.

The kind and amount of regeneration vary with level and size of piece. Grades of head varying from normal through inhibited types to headlessness occur. The rate of head formation, the frequency of normal heads and eyes, head size, and the distance of the pharynx from the head decrease along the anteroposterior axis, facts indicating the existence of an anteroposterior physiological gradient (first mentioned in 1911*b*). An increase in these matters in postpharyngeal regions shows the presence there of one or more zooids physiologically. The inability of pieces to form structures anterior to their own level unless some grade of head is first regenerated evidences head-dominance. The regulation of a piece to a whole consists essentially of the formation of a new individual beginning with the head and proceeding posteriorly under the control and dominance of the head. Newly hatched or newly regenerated normal worms have no posterior zooid. In normal worms the postpharyngeal region does not isolate as a zooid until a definite length is reached; but this happens at a very short length in headless and anophthalmic pieces, and these undergo fission if fed. Large worms consist of several zooids; starvation reduces the zooids and increases head dominance. The zooids disappear when the isolated postpharyngeal region regenerates a new head. Anything which increases the dominance of the head eliminates zooid formation, and vice versa. Decapitation induces fission in small animals.

1910*b*. Analysis of form regulation with the aid of anaesthetics. Biol. Bull., **18**:161–73.

1912*c*. Studies on the dynamics of morphogenesis, etc. IV. Certain dynamic factors in the regulatory morphogenesis of *Planaria dorotocephala* in relation to the axial gradient. Jour. Exper. Zoöl., **13**:103–52.

1913*a*. Studies on the dynamics of morphogenesis, etc. V. The relation between resistance to depressing agents and rate of metabolism in *Planaria dorotocephala* and its value as a method of investigation. Jour. Exper. Zoöl., **13**:103–52.

First full statement (1913*a*) of the technique and results of the direct and indirect susceptibility methods. In the higher concentrations of lethal agents death and disintegration begin at the anterior end and proceed posteriorly. Rate of disintegration is believed to parallel general metabolic rate, and consequently the disintegration gradient is interpreted to demonstrate the existence of a metabolic gradient along the main axis. In dilute concentrations a reverse disintegration gradient is obtained; regions of higher rate acclimate better when adequate time is allowed than regions of lower rate. The retarding effect of anesthetics on regeneration is greater the more posterior the level of the piece; this also indicates the presence of an axial factor, more fully analyzed below.

1913*c*. Studies on the dynamics of morphogenesis, etc. VI. The nature of the axial gradients in *Planaria* and their relation to antero-posterior dominance, polarity, and symmetry. Arch. f. Entw'mech., **37**:108–58.

Besides the principal anteroposterior gradient, *Planaria* has also lateromedial and ventrodorsal gradients in susceptibility to lethal solutions. The axiate organism in its simplest terms is a gradient in rate of reaction in a specific colloidal substratum. The region of highest rate dominates or controls other parts and determines the existence and maintenance of the gradient. The axial gradients are the basis of polarity and symmetry. The axial gradient theory alone explains how the organism as a whole can

have a polarity superimposed upon the varied polarities and orientations of its cells and organs. The apical end of the gradient represents, more nearly than any other part, self-differentiation in the specific reaction system of each kind of protoplasm; other parts are the result of correlative differentiation under the influence of the dominant part.

1913*b*. Certain dynamic factors in experimental reproduction and their significance for the problems of reproduction and development. Arch. f. Entw'mech., **35**:598–641.

1914*d*. Studies on the dynamics of morphogenesis, etc. VII. The stimulation of pieces by section in *Planaria dorotocephala*. Jour. Exper. Zoöl., **16**:413–41.

1914*f*. Studies on the dynamics of morphogenesis, etc. VIII. Dynamic factors in head-determination in *Planaria*. Jour. Exper. Zoöl., **17**:61–79.

1916*e*. Studies on the dynamics of morphogenesis, etc. IX. The control of head-form and head frequency in *Planaria* by means of potassium cyanide. Jour. Exper. Zoöl., **21**:101–26.

The metabolic rate of pieces of *Planaria* is increased by the mere cutting of them from the body; this increase is greater the shorter the piece and the more posterior its level in the first zooid. This stimulation lasts several hours and then gradually declines. Since this stimulation is greatest in those pieces which show the lowest head frequency in regeneration, there is evidently a relation between head frequency and stimulation of section. By cutting off short anterior pieces from long pieces at various intervals after the latter were cut, it is shown that whether or not a head shall form on a piece is determined within 6–8 hours after section, that is, during the period of greatest stimulation resulting from section. Inhibiting the stimulation of section by putting newly cut pieces into low concentrations of cyanide decreases the head frequency of anterior and increases that of posterior pieces of the first zooid. These facts indicate an antagonism between the activity of the blastema at the anterior cut surface and the old tissue of the piece. If the former (x) dominates, head frequency is high; if the latter (y) dominates, head frequency is low. Head frequency therefore depends on the ratio x/y and can be altered by experimental alteration of either x or y. Cyanide directly inhibits x in anterior pieces and hence decreases head frequency; it prevents the stimulation of y in posterior pieces and hence increases head frequency.

1911*c*. A study of senescence and rejuvenescence based on experiments with *Planaria dorotocephala*. Arch. f. Entw'mech., **31**:537–616.

1912*a*. The process of reproduction in organisms. Biol. Bull., **23**:1–39.

1913*d*. The asexual cycle of *Planaria velata* in relation to senescence and rejuvenescence. Biol. Bull., **25**:181–203.

1914*c*. Starvation, rejuvenescence, and acclimation in *Planaria dorotocephala*. Arch. f. Entw'mech., **38**:418–46.

1914*e*. Asexual breeding and prevention of senescence in *Planaria velata*. Biol. Bull., **23**:1–39.

1915*c*. Senescence and rejuvenescence. Chicago: University of Chicago Press. Pp. 481.

1916*b*. Age cycles and other periodicities in organisms. Proc. Amer. Philos. Soc., **55**: 330–39.

1916*d*. Age cycles and other periodicities in organisms. Abstract of 1916*b*. Science, **43**:724–25.

1918. Physiological senscence in Hydromedusae. Biol. Bull., **34**:49–63.

The theory of, and experiments on, senescence and rejuvenescence are summarized in 1915*c*. The organism is a machine constructed *by* functioning, not *for* function; function and structure are inseparable. Growth and differentiation entail the accumulation of visible structural materials; in lower forms they are reversible processes, and reduction and dedifferentiation are accompanied by removal of structure. Senescence is primarily a decrease in rate of dynamic processes conditioned by the accumulation, differentiation, and other associated changes of the material of the colloid substratum. Rejuvenescence is an increase in rate of dynamic processes conditioned by the changes in the colloid substratum in reduction and dedifferentiation. The direct susceptibility method is presented as a means of measuring roughly the rate of dynamic processes. By this means metabolic rate is found to be higher the younger (or smaller) the animal in various Turbellaria, hydroids, hydromedusae, and ctenophores; higher in the animals resulting from regenerated pieces than in the animals from which the pieces were taken; and higher the more normal the regenerate, i.e., the greater the degree of reconstitution of the piece. The products of fission and budding also resemble young animals metabolically. *Planaria velata* reproduces regularly by fragmenting into small pieces, which encyst and emerge as complete tiny worms. This species can apparently exist indefinitely by this asexual cycle without senescence. The reconstitution involved in regeneration and asexual reproduction are thus rejuvenating. In planarians starvation and accompanying reduction result in increased susceptibility; hence a metabolic condition resembling youth. *P. velata* will exist indefinitely in a youthful state if prevented from growing by properly limited food supply. The axial gradient and the role of physiological isolation from a dominant region in asexual reproduction are discussed. Senescence in plants and lower animals results in decreased dominance of the apical end; hence agamic reproduction with accompanying rejuvenescence. Senescence in man and higher animals appears also to consist of accumulation of structural materials, decline of water content, decrease in growth rate, and decline of metabolic rate; hibernation and starvation probably have some rejuvenating action here. Gamete formation is a process of specialization and differentiation; hence, physiologically gametes are in a state of advanced senescence with accompanying low metabolic rate. Gametes generally form toward the end of the life-cycle, usually after a period of vegetative or asexual reproduction, or of growth. Fertilization and early development are rejuvenating processes. Agamic and gametic reproduction are essentially similar, but the gametes are so far specialized that the peculiarities associated with fertilization are necessary for the initiation in them of rejuvenescence. Senescence is a characteristic and necessary feature of the vegetative life of organisms; rejuvenescence is usually associated with reproduction. In lower forms senescence may be balanced by the rejuvenescence of reproduction, so that there is no progressive senescence or death, and life consists of brief alternating periods of progression and regression; in higher forms senescence of the individual leads to death.

1915*a*. A dynamic conception of the organic individual. Proc. Nat. Acad. Sci., **1**:164–72.

1915*d*. Individuality in organisms. Chicago: University of Chicago Press. Pp 213.

1916*a*. The basis of physiological individuality in organisms. Science, N.S., **43**:511–23.

Definite form, size, structural pattern, and correlation in activities characterize the organic individual. Particulate and vitalistic theories of individuality or theories based on transport of chemical substances fail to touch the essential problem, the order and unity of the organism. The transmission of dynamic change is presented as the basis of organic individuality. Dynamic change in the last analysis results from the action of external forces on protoplasm; if such action is directed and continued, a more or less permanent gradient of response will be produced in a mass of undifferentiated proto-

plasm. Such a gradient in the transmission of excitation represents the simplest expression of unity and order in protoplasm and is the simplest axis of polarity and symmetry. Along such a metabolic gradient a relation of dominance and subordination exists, the most active region dominating the others because of its greater speed of reactivity. The evidence for the existence of such metabolic gradients (as tested by susceptibility to lethal agents) in a large number of plants and lower animals and embryos is presented. The facts of regeneration show that the apical region of plants and animals is independent of, and controls, other levels along the major axes to a certain distance. Regeneration is fundamentally the same as embryonic development, consisting in the formation of the apical dominant region first, and then the appearance of other parts in correlation with this. The spatial relations of parts of organisms can be experimentally altered by altering the range of apical dominance, through increasing length of axis, decrease in apical activity, decrease in conductivity, or increase in independence of subordinate parts. Parts thus beyond the range of dominance (physiologically isolated) may produce new gradients and so new individuals. Existing gradients can be abolished; and new gradients, and hence new individuals, established by experimental means. Dominance depends on transmitted change, not on transported substances, and generally increases in range with age, because of the improvement in conducting paths with age.

1914*a*. The axial gradient in ciliate Infusoria. Biol. Bull., **26**:36–54.

1914*b*. Susceptibility gradients in animals. Science, N.S., **39**:73–76.

1917*b*. The gradient in susceptibility to cyanides in the meridional conducting path of the ctenaphore, *Mnemiopsis*. Amer. Jour. Physiol., **43**:87–112.

1919*b*. With L. H. HYMAN. The axial gradients in Hydrozoa. I. Hydra. Biol. Bull., **36**:183–223.

1919*d*. The axial gradients in Hydrozoa. II. Susceptibility in relation to physiological axes, regions of colony, and stages of development. Biol. Bull., **37**:101–25.

Apicobasal disintegration gradients in lethal solutions are reported for a variety of Protozoa, various embryos, hydra and other hydroids, and hydroid colonies; a centroperipheral gradient was found along the nerve of the spider-crab claw; and an aboral-oral gradient along the plate-row of *Mnemiopsis*. Local activities may cause secondary regions of high susceptibility at the stalk of hydra. Lethal agents not only slow down the plates of ctenophores but diminish the transmission, so that the oral parts of plate rows may become physiologically isolated and beat independently.

1915*b*. Axial gradients in the early development of the starfish. Amer. Jour. Physiol., **37**:203–19.

1916*c*. Axial susceptibility gradients in the early development of the sea-urchin. Biol. Bull., **30**:391–405.

1916*g*. Experimental control and modification of larval development in the sea-urchin in relation to the axial gradients. Jour. Morph., **28**:65–133.

1917*c*. Differential susceptibility and differential inhibition in the development of polychaete annelids. Jour. Morph., **30**:1–63.

1924*a*. With A. W. BELLAMY. Susceptibility in amphibian development. Proc. Roy. Soc. London, B, **96**:132–45.

In starfish and sea-urchin eggs, blastulae, and gastrulae and in the early embryos of annelids a gradient is found from animal to vegetative pole; later in annelids and amphibians the posterior growing region becomes prominent as a secondary gradient of activity. In proper concentrations, toxic agents modify development along the lines

of either differential inhibition or differential recovery. The former gives microcephalic forms with suppression of the oral lobe in urchins, closer approximation or fusion of the pluteus arms, and progressive obliteration of anteroposterior and mediolateral differentiation; in annelids head and posterior region are inhibited. Differential recovery in sea-urchin larvae results in an increased oral lobe, divergence of the arms, and overdevelopment of anterior and median, as compared to lateral and posterior, parts. These results show that the spatial arrangement and the relative sizes and proportions of parts are controlled by the axial gradients.

1916*f*. Axial ausceptibility gradients in algae. Bot. Gaz., **62**:89–114.

1916*h*. Further observations on axial susceptibility gradients in algae. Biol. Bull., **31**: 419–40.

1917*a*. Susceptibility gradients in the hairs of certain marine algae. Biol. Bull., **32**:75–92.

1917*c*. Experimental alteration of the axial gradient in the alga, *Griffithsia bornetiana*. Biol. Bull., **32**:213–33.

1919*c*. A study of susceptibility in some Puget Sound algae. Publ. Puget Sound Biol. Sta. 2, No. 48, pp. 249–67.

In a variety of algae of the axiated type, apicobasal disintegration or staining gradients are demonstrated when they are placed in lethal or various colored solutions. A gradient in permeability to the agents is also present. The hairs of various marine algae show an apicobasal gradient when the growth is apical, a basoapical gradient when growth is basal. Obliteration or reversal of the gradient is induced in some cases in lower concentrations of the agents. *Griffithsia*, when confined in a small volume of water, may show a reversal of the normal basipetal gradient and may break up into individual cells. Such cells may grow out new filaments; the apical end of the cell in such cases is whatever end was the most susceptible regardless of the original polarity.

1919*a*. Demonstration of the axial gradients by means of potassium permanganate. Biol. Bull., **36**:133–47.

1921*d*. The axial gradients of Hydrozoa. IV. Axial gradients in rate and amount of reduction of potassium permanganate in various hydroids and medusae. Biol. Bull., **41**:78–97.

Various organisms placed in weak permanganate solutions show a gradation in color reduction from anterior or oral to posterior or basal end. Medusae give the greatest reduction in marginal and oral regions.

1919*f*. Physiological isolation by low temperature in *Bryophyllum* and other plants. Science, N.S., **50**:362–65.

1920*d*. With A. W. Bellamy. Physiological isolation by low temperature in *Bryophyllum*. Bot. Gaz., **70**:249–67.

The growing tip of plants dominates lower levels and prevents vegetative growth within a certain distance. A cold block placed below the dominant region does no damage but impedes transmission; it induces the outgrowth of axillary buds in bean seedlings and the formation of plants at the runner tips in *Saxifraga* at much shorter than normal length. A cold block around the petiole of *Bryophyllum* leaves induces the outgrowth of plantlets in the angles of the leaf scallops.

1919*b*. A comparative study of carbon dioxide production during starvation in *Planaria*. Amer. Jour. Physiol., **48**:241–57.

1919*c*. The effects of cyanide on carbon dioxide production and on susceptibility to lack of oxygen in *Planaria dorotocephala*. Amer. Jour. Physiol., **48**:372–95.

1919*e*. Susceptibility to lack of oxygen during starvation in *Planaria*. Amer. Jour. Physiol., **49**:403–19.

1920*a*. With H. L. ROBBINS. Carbon dioxide production in relation to regeneration in *Planaria dorotocephala*. Biol. Bull., **38**:103–22.

Carbon dioxide production, measured by the rate of color change of phenol red, is decreased after exposure to cyanide, decreased in early starvation increased in prolonged starvation, increased by regeneration, and increased temporarily, greatest in the mouth region, by cutting pieces. The death of *Planaria* in cyanide is accelerated by lack of oxygen; an additive effect is indicated. During starvation the susceptibility of the body wall to lack of oxygen increases progressively up to at least 4 months. The reaction to lack of oxygen of such starved animals is the same as that of young fed animals of the same size.

1920*b*. Studies on the dynamics of morphogenesis, etc. X. The head frequency in *Planaria dorotocephala* in relation to age, nutrition, and motor activity. Jour. Exper. Zoöl., **30**:403–18.

1921*c*. Studies on the dynamics of morphogenesis, etc. XI. Physiological factors in the development of the planaria head. Jour. Exper. Zoöl., **33**:409–33.

Further exposition of the role in head regeneration of the relative rates of activity of the anterior blastema (x) and the old piece (y). Head frequency is lower in young than in old, in starved than in fed, and in pieces kept moving than in quiescent pieces, because the rate of y is relatively higher in the first of each of the paired conditions mentioned. The median region of the head is more susceptible than the lateral regions, and inhibited types of head may show differential recovery after a time with excessive outgrowth of the median region.

1920*c*. Some considerations concerning the nature and origin of physiological gradients. Biol. Bull., **39**:147–87.

1921*b*. Certain aspects of the problem of physiological correlation. Amer. Jour. Bot., **8**:286–95.

1921*e*. Le Problème de l'intégration physiologique. Scientia, **30**:115–26.

1923*c*. Physiological polarity and symmetry in relation to heredity. Genetics, **8**:336–54.

1924*b*. The problem of pattern in organisms. I. General survey. Amer. Nat., **58**:115–26.

1924*e*. The problem of pattern in organisms. II. The physiological gradients. Amer. Nat., **58**:322–36.

1925*d*. The physiological significance of the cephalocaudal differential in vertebrate development. Anat. Rec., **31**:369–83.

The chief characteristic of the organism is integration. The problem of organismic integration is one both of pattern and material. The chief pattern of organisms, the axiate pattern, is quantitative and consists in simplest terms of a gradient in rate of the main physiological processes. The various lines of evidence in support of this view are reviewed: evidence from susceptibility differences along the axis of numerous embryos, plants, and animals; of staining and reduction gradients in colored substances; of modifications of development in various agents; differences in electric potential along the axis; axial differences in oxygen consumption and carbon dioxide production. Axiate pattern in the last analysis arises from the differential action of the external world on

protoplasm. The gradients represent physiological conditions under which the hereditary mechanism gives rise to an axiate pattern, and are the physiological basis of polarity and symmetry. They are chiefly significant in initiating orderly series of development events in definite directions. In vertebrate development there is a primary cephalocaudal differential; but later, as in other segmented animals, i.e., annelids, the posterior region shows a caudocephalic gradient; this eventually in vertebrates comes under the control of the head. While the gradients arise in relation to environmental differentials, the specific differentiation and organization resulting from development depend upon the hereditary constitution of the protoplasm concerned.

1921*a*. The origin and development of the nervous system from a physiological viewpoint. Chicago: University of Chicago Press. Pp. xvii+296.

The earlier chapters treat in detail the same topics as above: the problem of pattern, the quantitative gradient as the simplest form of axiate pattern, the evidence for the existence of physiological gradients, dominance and subordination as results of the gradient. The nervous system is the chief morphological and physiological expression of the gradient; the chief aggregations of nervous tissue develop at the high end of the gradient, and further differentiation then proceeds posteriorly along the gradient. Functionally, the simple gradient is an excitation-transmission gradient along which nervous function gradually develops, as seen in the ctenophore plate-row. Other transmissive gradients are exemplified by the heart and digestive tube. Differences in electric potential are characteristic features of the gradients; it is suggested that the outgrowths of neurones and formation of paths in the development of the nervous system are directed by such electric differences. The reflex arc also appears to be related to pattern. Early reflexes are effective mostly in a direction down the gradient. In vertebrate evolution there seems to be an increasing tendency toward upward paths. During vertebrate development the appearance of a secondary caudocephalic gradient in posterior regions may determine short, upward paths.

1923*d*. The axial gradients in Hydrozoa. V. Experimental axial transformations in hydroids. Biol. Bull., **45**:181–99.

1923*e*. The general relation between susceptibility and physiological condition. Arch. Int. Med., **32**:647–62.

1923*f*. Modification of hydroid development through differential susceptibility. Abstract. Anat. Rec., **26**:362–63.

1924*c*. Modification of development in relation to differential susceptibility. Amer. Nat., **58**:237–53.

1925*b*. Quantitative factors in the susceptibility of living cells to external agents. Radiology, **4**:21–29.

1925*c*. The axial gradients in Hydrozoa. VII. Modification of development through differential susceptibility. Biol. Bull., **48**:176–99.

In several general papers the direct and indirect susceptibility methods and their application in experimental work are again reviewed. The basis of the direct susceptibility method is explained thus: "To disturbances producing in any manner alteration beyond the limits of equilibration or tolerance of the system and therefore leading to disruption or irreversible change within a measurable period of time, the susceptibility must vary directly though not necessarily proportionally to the rate of changes characteristic of the system." Thus, a great variety of agents and conditions act in a nonspecific manner and give evidence for the existence of a quantitative gradation in rate of activity along the main axis. Modification of development by differential action of inhibiting agents in relation to the gradient is reviewed and new evidence from hydroids

brought forward that the higher levels of the gradient are most inhibited by such agents when acting directly. In hydroids, slightly depressing conditions induce stolons anywhere in the colony and cause hydranth buds to transform into stolons. Planulae are modified from the elongated to a spherical apparently anaxial form; they may recover and then show several polarities, as evidenced by stolon outgrowth. Stolons represent a lower metabolic level than hydranths.

1923*a*. The axial gradients in *Corymorpha palma*. Abstract. Anat. Rec., **24**:392.

1925*a*. The axial gradients in Hydrozoa. VI. Embryonic development of hydroids. Biol. Bull., **48**:19–36.

1926*a*. With E. DEVINEY. Contributions to the physiology of *Paramecium caudatum*. Jour. Exper. Zoöl., **43**:257–312.

1926*b*. With L. H. HYMAN. Studies on the axial gradients of *Corymorpha palma*. I. Respiratory, electric, and reconstitutional gradients. Biol. Generalis, **2**:355–74.

1926*d*. Studies on the axial gradients of *Corymorpha palma*. II. Differential susceptibility, penetration, and oxidation-reduction in development and reconstitution. Biol. Generalis, **2**:609–30.

Further evidence for axial gradients, using *Paramecium* and hydroid embryos and adults, by means of differential susceptibility, staining and reduction gradients, susceptibility to ultraviolet radiations and ordinary light after sensitization, etc. In *Corymorpha*, axial differences in electric potential, oxygen consumption, carbon dioxide production, and rate and type of reconstitution are reported.

1924*d*. Physiological foundations of behavior. New York: Henry Holt & Co. Pp. xii+330.

1926*c*. Behavior origins from a physiologic point of view. Arch. Neurol. and Psychiat., **15**:173–84. (Read before the Fifty-first Annual Meeting of the American Neurological Association, Washington, May, 1925.)

1927*a*. The individual and environment from a physiological viewpoint. Article in The child, the clinic, and the court, pp. 126–55. New York: New Republic, Inc.

1928*c*. Biological foundations of social integration. Publ. Amer. Sociol. Soc., **22**:26–42.

A consideration of behavior in the broad sense of reaction to environment. The individual is a behavior pattern in a specific protoplasm. The types of patterns found in organisms—spherical, radial, and bilateral—are reviewed in relation to growth and development in plants and animals. Bilateral symmetry gives the greatest possibilities for differentiation and specialization. The axiate type of pattern (radial, bilateral) originates as an excitation-transmission gradient in protoplasm in response to directed external stimuli. The evidence for the existence of such gradients in relation to the primary axis is reviewed. This evidence comprises: structural axial differences, axial differences in normal development, differential modifications of development by the action of external agents, differential death in various solutions and under various conditions, axial differences in electric potential and respiratory rate, staining and reduction differences. The fact that development can be profoundly modified by the differential action of inhibiting agents with respect to the axial gradient shows the importance of the gradients in normal development. The simple excitation-transmission gradient determines the degree and extent of integration. A relation of dominance and subordination is established—the high, or head, end of the gradient dominates the rest of the axis and acts as pacemaker. Blocking of the transmission or decrease in its effectiveness with distance results in physiological isolation. Twinning and duplication of parts are examples of physiological isolation. The organism evolves as a behavior

mechanism, since life is a continuous process of equilibration. Evolution is concerned not with morphological characters but with physiological mechanisms of integration and equilibration. Development is a series of modifications of pattern, and the normal patterns can be readily modified by acting on the gradients. Both fixed and modifiable behavior represent the potentialities of the specific protoplasm. The more fixed behavior is more correlated with the primary physiological gradient than is the more modifiable behavior. In evolution the simple behavior type, dependent on anteroposterior dominance, is replaced by central control; in the higher animals, by the cortex, which is not axiate, receives and correlates impulses from all parts of the body, and is the basis of the great modifiability of behavior seen in higher animals. Human social organization is compared to organismic organization. It consists of a relation of dominance (government) and subordination (the governed). Social integration depends on a dynamic correlation of a transmissive nature among the governed. Leadership or dominance originates directly in environmental conditions and is alterable by environment. Societies and nations are integrated by dominant ideas and are altered by this integration. The lower forms of social integration resemble the lower types of organismic integration in that there is an autocratic relation of dominance and subordination. In higher animals and in higher forms of societies (democracy) there is an escape from this autocracy by means of representative government, corresponding to the development of the cortex in animals.

1926*e*. Experimental control of polarity in *Corymorpha palma*. Proc. Soc. Exper. Biol. and Med., **23**:789–90.

1926*g*. Studies on the axial gradients of *Corymorpha palma*. III. Control and modification of polarity and symmetry in reconstitution by differential exposure. Biol. Generalis, **2**:771–98.

Regenerating pieces of *Corymorpha* give rise to unipolar, bipolar, and multipolar forms with one, two, or more complete or partial hydranths at one or both ends. The frequency of the various forms varies with level and length of piece. Hydranths arise at points of high metabolic rate; regions in contact tend to develop basal ends. The environmental differential between free surfaces and those in contact thus controls the result and the frequency of the various types of regenerates, and origin of new polarities can be altered by means of this factor.

1926*f*. Physiological control: its protoplasmic basis and its development. Ann. Clin. Med., **4**:884–99.

The gradient as the primary expression of polarity and symmetry and the evidence for gradients from differential susceptibility and modification of development are reviewed. The gradient as the basis of physiological control is discussed; pacemakers are examples of control by the high end of the gradient over lower levels. Neoplasms are considered to represent escapes from normal physiological control.

1924*f*. Experimental regression and redevelopment in *Corymorpha palma*. Abstract of 1927*a*. Anat. Rec., **29**:98.

1927*a*. Modification of polarity and symmetry in *Corymorpha palma* by means of inhibiting conditions and differential exposure. I. Forms resulting from modification. Jour. Exper. Zoöl., **47**:343–83.

1927*b*. Modification of polarity and symmetry, etc. II. Changes in form frequency. Jour. Exper. Zoöl., **48**:197–221.

Temporary subjection of reconstituting pieces to inhibiting or depressing agents or conditions more or less obliterates the original polarity; and on return to normal condi-

tions, new polarities arise in such pieces without regard to the original polarity or the cut ends but with reference to the exposed surface. Single or multiple partial or complete hydranths grow out from the exposed surface; single or multiple basal ends, from the surface in contact. The frequency of the various types of regenerates is tabulated. Inhibiting agents decrease the frequency of bipolar forms and increase that of heteropolar forms. Less extreme degrees of inhibition increase unipolar regenerates; more extreme increase multipolar ones.

1923*b*. Experimental eliminations of the larval stage in an ascidian. Abstract, American Association of Anatomists. Anat. Rec., **25**:122–23.

1927*c*. Developmental modification and elimination of the larval stage in the ascidian, *Corella willmeriana*. Jour. Morph., **44**:467–514.

When the eggs of *Corella*, which normally develop in the atrial chamber of the parent at a pH below that of normal sea water, are removed to normal sea water at early stages, larval development is differentially inhibited, the tail most, the dorsal region less. The regions most inhibited are those showing the highest reducing power in potassium permanganate solutions. The effective natural factor is the CO_2 content of the atrial chamber; but both normal and inhibited development can be produced by other means.

1928*a*. Experimental transformations of bipolar forms in *Corymorpha palma*. Biol. Bull., **54**:1–14.

Bipolar forms with a hydranth at each end regress when placed in inhibiting solutions for one or two days, and may then regenerate in normal sea water into a unipolar form. Bipolar forms under depressing conditions may grow out a basal end laterally between the two hydranths, which then face one way, as a double-headed unipolar form.

1928*b*. Physiological polarity and dominance in the holdfast system of *Corymorpha*. Biol. Bull., **54**:15–35.

Observations on the formation, structure, and behavior of holdfasts; they show a susceptibility gradient from tip to base and apparently develop under conditions of physiological isolation.

1928*d*. Axial development in aggregations of dissociated cells from *Corymorpha palma*. Physiol. Zoöl., **1**:419–61.

Cells and cell groups of the naked stem region, obtained by grinding with sand and then straining through bolting cloth, aggregate into masses which may develop into single or doubled hydranths with stems or imperfect hydranths or irregular tentacles. The polarity is determined by the environmental differential between the exposed and the attached surface of the masses.

1927*d*. Experimental localization of new axes in *Corymorpha palma* without obliteration of the original polarity. Biol. Bull., **53**:469–80.

1929*b*. Lateral grafts and incisions as organizers in the hydroid *Corymorpha*. Physiol. Zoöl., **2**:342–74.

1932*b*. Determination of new axes by lateral grafts and incisions in *Corymorpha* in relation to the gradient and dominance. Physiol. Zoöl., **5**:593–627.

1935*c*. Dominance of hydranths induced by grafts in *Corymorpha*. Jour. Exper. Zoöl., **71**:375–87.

A lacerated area in the side of the *Corymorpha* stem may grow out into a hydranth opposite which a basal end may develop, the whole forming an axis at right angles to the original axis. Small grafts implanted into incisions in the stem often grow out into a

hydranth composed of both graft and host tissue. The removal of the host hydranth is essential, as its presence inhibits the graft. These grafts are more successful the more distal the region of the stem from which they come and the greater their distance from the host hydranth. The hydranth induced by such grafts dominates a certain length of host stem distal and proximal to it, as indicated by inhibition of regeneration on the host stem, reversal of polarity, or determination of new polarity in the host stem.

1928*e*. The physiological gradients. Sammelreferat. Protoplasma, **5**:447–76.

1929*c*. Physiological dominance and physiological isolation in development and reconstitution. Arch. f. Entw'mech., **117**:21–66.

General reviews of the gradients, the various methods of demonstrating them, differential modifications of development, dominance and subordination in plants and animals, physiological isolation and its relation to asexual reproduction. The amphibian organizer is probably simply a region of high rate of chemical activity which acts by increasing the metabolic rate of other tissues to the point where they can differentiate into nervous system.

1931*b*. The organism and environment. Peking Nat. Hist. Bull., **5**, Part IV: 33–36.

A lecture delivered in China reviewing briefly organismic patterns, their relation to environment, polarity as a quantitative gradient, experimental alterations of the gradient, and determination of polarity and new axes. The essential features of the organism are regarded as a reaction of protoplasm to environment.

1929*a*. Senescence and rejuvenescence from a biological viewpoint. Bull. N.Y. Acad. Med., **5**:111–13.

1930*a*. Senescence and rejuvenescence from a biological viewpoint. The Harvey Lectures, Ser. 24, pp. 25–44.

A condensed restatement of the material of 1915*c*.

1930*b*. The susceptibility of *Planaria* to potassium cyanide in relation to hydrion concentration and to certain changes in salt content of the medium. Physiol. Zoöl., **3**:90–134.

Differences in the pH, salt content, and osmotic pressure of the medium alter the time of death of *Planaria dorotocephala* and *P. maculata* in cyanide solutions, but in all cases the same susceptibility gradient is observed. The rate of progress of death and presence or absence of disintegration vary greatly with the medium.

1930*c*. With K. M. Beyer. Reconstitution of lateral pieces of *Planaria dorotocephala* and *P. maculata*. Physiol. Zoöl., **3**:342–65.

In lateral halves of transverse pieces the original polarity persists in almost all pieces; but in longitudinal strips taken lateral to the ventral nerve cords, one or more heads develop on the median cut surface at right angles to the original polarity in the great majority of cases.

1931*a*. Experimental modification of the scale of organization in the reconstitution of *Tubularia*. Physiol. Zoöl., **4**:165–88.

In short pieces, exposure to cyanide solutions decreases the length of the hydranth primordium and so increases the frequency of regenerated hydranths with stems and inhibits the formation of aboral hydranths.

1932*b*. Experimental studies on a Japanese planarian. I. Fission and differential susceptibility. Sci. Repts., Tohoku Univ., Ser. 4, **7**:313–45.

In *Planaria gonocephala* rapid growth and decapitation accelerate fission; a third zooid is well developed and the posterior fission product may soon divide again. The anteroposterior susceptibility gradient shows a secondary reversed region in the posterior end. Experiments on the differential effect of cyanide on the two ends of regenerating pieces are presented.

1933*a*. The swimming plate rows of the ctenophore, *Pleurobrachia*, as gradients; with comparative data on other forms. Jour. Comp. Neurol., **57**:199–252.

A relation of dominance and subordination exists along each plate-row from aboral to oral end. The aboral end dominates; but any level, if stimulated, can initiate rhythmic impulses and dominate temporarily. Transmission may occur in both directions along a row, and the stronger impulse dominates. The inactivity of a single plate can block the impulse; hence the impulse must be reinitiated or reinforced at each plate. Reversed locomotion is brought about by reversed transmission. Toxic agents show an aboral-oral susceptibility gradient along the plate row; this is interpreted as an excitation-transmission gradient behaving in an all-or-none fashion.

1933*b*. Reconstitution in *Haliclystus auricula* Clark. Sci. Repts., Tohoku Univ., Ser. 4, **8**:75–106.

Removal of distal parts is followed by regeneration of tentacles and sense organs and reorganization of the old tissue. Distal pieces bearing sense organs regenerate but little. After oblique cuts, the presence or regeneration of distal sense organs inhibits regeneration at more proximal levels. The distal part of the stalk can reorganize into an umbrella. In toxic solutions the umbrellar margin and manubrial end are the most susceptible and susceptibility decreases proximally along the umbrella.

1933*c*. With Y. Watanabe. The longitudinal gradient in *Stylochus ijimai:* with a critical discussion. Physiol. Zoöl., **6**:542–91.

The carbon dioxide production of an axial series of pieces gives a U-shaped gradient, varying somewhat with sexual condition. Carbon dioxide production varies inversely with age. Late embryos have a simple apicobasal susceptibility and reduction gradient, while that of adults is U-shaped, agreeing with the carbon dioxide determinations. Criticisms are answered and discussed.

1934*a*. Differential reduction of methylene blue by living organisms. Proc. Soc. Exper. Biol. and Med., **32**:34–36.

1934*b*. The differential reduction of methylene blue by *Paramecium* and other ciliates. Protoplasma, **22**:377–94.

1935*b*. With Y. Watanabe. Differential reduction of methylene blue by *Corymorpha palma*. Physiol. Zoöl., **8**:395–416.

1936*c*. Differential reduction of vital dyes in the early development of echinoderms. Arch. f. Entw'mech., **135**:426–56.

1936*b*. With O. Rulon. Differential reduction of vital dyes in certain oligochaetes. Jour. Exper. Zoöl., **74**:427–48.

The reduction of methylene blue and janus green in various animals and embryos gives gradients similar to those obtained by other methods. Pieces of *Corymorpha* stems show an apicobasal reduction gradient 5–8 hours after section; irregular results obtain shortly after section. The animal-vegetal reduction gradient of early echinoderm embryos is later disturbed by an increased reducing-power of the primary mesenchyme and the entodermal region before and during gastrulation.

1936*a*. With F. J. WIERCINSKI. Differential susceptibility of living organisms to supersonic vibrations. Science, **83**:604–6.

Supersonic vibrations, a primarily mechanical factor, produce disintegration gradients in *Planaria*, *Tubifex*, and *Hydra*, which are apparently the same as those observed with other agents.

1935*a*. With Y. WATANABE. The head frequency gradient in *Euplanaria dorotocephala*. Physiol. Zoöl., **8**:1–40.

At a given level the head frequency decreases with decreasing length of piece, and in short pieces decreases from anterior to posterior end of the first zooid, increasing again in the second zooid. The head frequency rises with delay in cutting short anterior or posterior pieces from the ends of long pieces. The removal of the anterior end of acephalic pieces is followed by regeneration of a normal head, but not if a posterior cut surface is also made. These facts indicate an inhibition of head regeneration emanating from the posterior cut surface, probably from severed nerve paths. Transverse half-cuts with or without removal of tissue near an anterior cut surface retard but do not inhibit head regeneration of that side.

1936*d*. A contribution to the physiology of exogastrulation in echinoderms. Arch. f. Entw'mech., **135**:457–93.

The exogastrulae of echinoderms, produced chiefly by LiCl, are discussed from the viewpoint of physiological gradients. Exogastrulation is the result of a high degree of differential inhibition applied at the beginning of development; entoderm and mesenchyme increase at the expense of ectoderm. Applied at late blastula stages, inhibiting agents inhibit the formation of mesenchyme and entoderm, acting directly on the gastric region. In exogastrulae the staining gradients to dyes are inhibited or reversed. Differential recovery expresses itself in thickening and outgrowth of the apical ectoderm and in secondary invaginations.

1937. With O. RULON. Observations and experiments on developmental pattern in *Pelmatohydra oligactis*. Physiol. Zoöl., **10**:1–13.

The pattern of development of tentacles on buds is correlated with that of the parent. Pieces with different anterior levels decrease in regeneration rate and number of regenerated tentacles the more posterior the anterior cut. Buds dominate a certain amount of the adjacent column and inhibit anterior regeneration from cut surfaces near them.

REVIEWS

1906. E. G. CONKLIN. The organization and cell lineage of the ascidian egg (Jour. Acad. Nat. Sci. Phil., Ser. 2, **13**. Part I [1905]: 1–127). Science, N.S., **23**:340–44.

1907. T. H. MORGAN. Experimental zoölogy. Science, N.S., **26**:824–29.

1913. W. ROUX, in Verbindung mit PROFESSOR C. COWENS, PROFESSOR A. FISCHEL und PROFESSOR E. KUSTER. Terminologie der Entwicklungsmechanik der Tiere und Pflanzen. Science, N.S., **37**:668–69.

1918. J. LOEB. The organism as a whole. Bot. Gaz., **65**:274–80.

1925. J. KEMSTER et F. PREVOST. La Matière vivante. Jour. Amer. Med. Assoc., **84**:808.

THE FRAGMENTATION OF NAIS PARAGUAYENSIS

(Two figures)

LIBBIE H. HYMAN
Laboratory of Experimental Biology, American Museum of Natural History

THIS report is offered as a contribution to Child's theory of dominance and subordination and physiological isolation in relation to asexual reproduction, among the most fruitful of the conceptions with which he has enriched biology.

INTRODUCTION

1. *The experimental animal.*—The oligochaete which forms the subject of these experiments was accidentally discovered three years ago in aquariums in the Laboratory of Experimental Biology, American Museum of Natural History. My attention was attracted by the fact that fission planes were never seen in the worms although they were obviously multiplying by some asexual method. The worm evidently belonged to the family Naididae, notable among oligochaetes for their method of asexual multiplication by the formation of fission planes partitioning the worm into a chain of two or more zooids, each of which alters into a complete worm with fully developed head and posterior end before breaking loose from the chain. In this particular naid, fission planes were never present; but the finding of many regenerating fragments lead to the suspicion that the animal must reproduce by breaking up into pieces. Accordingly, a worm was isolated into a small dish of water and was found on the next day divided into several regenerating pieces. Other worms were then isolated with the same result. It was thus established that this species of Naididae does not conform to the method of asexual reproduction by fission planes characteristic of the family but instead multiplies by fragmentation. The experimental possibilities of the species were at once perceived, and the animal has been under experimentation continuously since its discovery.

The worm was identified as *Nais paraguayensis* Michaelsen, 1905, known from South America and India. The species is not native to the United States and was probably introduced into the laboratory aquariums with South American plants or fish. The absence of fission planes was noted by the discoverer and other taxonomists on oligochaetes, who suspected reproduction by fragmentation, as mentioned in Stephenson's book (1930, p. 538). The fact of fragmentation was established by Aiyer (1924), who, however, is mistaken in supposing that the worm simply divides in two. Stephenson mentions several other oligochaetes supposed to undergo fragmentation as a regular mode of asexual reproduction, but consultation of the original papers failed to yield any convincing evidence in these cases. Consequently, *N. paraguayensis* constitutes the one well-established case among the oligochaetes of fragmentation as a regular method of asexual reproduction.

It was found that the worm can be cultivated easily and enormous numbers obtained in a very short time. The method of culture is given in detail at the end of the paper.

2. *Normal process of fragmentation.*—When adequately supplied with food, *N. paraguayensis* grows until it reaches a length of 30–40 mm. and then spontaneously frag-

ments into several pieces, usually six to eight, sometimes more. The places at which fragmentation is to occur are not detectable by microscopic examination of the worms. I have not personally witnessed the fragmentation; but a student of Dr. J. W. Buchanan, of Northwestern University, has seen three worms fragment. For some hours preceding the event, the worm coils and uncoils restlessly, but finally twists into a rather tight coil and shortly thereafter constricts and pulls apart. In the observed cases the worms divided into two fragments only. Fragmentation apparently occurs chiefly at night, whether the animals are illuminated or kept in darkness. Further observations on these matters are planned for the future.

The fragments are commonly of approximately equal length and segment number. The head fragment is always longer absolutely and contains more segments than the trunk fragments. It commonly consists of twenty to forty segments in addition to the five specialized head segments. The trunk fragments are of about equal length as a rule, consisting of ten to twenty-five segments. The tail fragment, although commonly but little longer absolutely than the trunk fragments, always contains many more segments than they, because of the gradual decrease in segment size toward the tail. There are usually thirty-five to fifty countable segments in the tail piece, as well as several indefinite segments in process of differentiation. No definite relation between body level and fragment length, beyond those just mentioned, has been noticed; but, in fact, it is rarely possible to know just where in the body axis a given fragment belongs. Figure 1 illustrates the fragments obtained from two worms.

Fragmentation cannot be induced by mechanical means, such as violently squirting the worms in and out of a pipette.

3. *Regeneration.*—Very little study was made of the regeneration process itself. Within a day or two at temperatures of 25°–30° C., each fragment has regenerated a complete new head of five segments and an anal segment. There follows the usual reorganization of the old segments behind the new head into the morphology typical of the postcephalic region (Hyman, 1916). New segments quickly differentiate anterior to the anal segment, and soon the regenerated worm has a long, new tail distinguishable for a few days from the old segments of the original fragment by the light color and smaller size of its segments. Soon the limits of the original fragment are no longer distinguishable; and in 4 or 5 days at room temperature, if food is abundant, the regenerated worms have grown to full length and soon fragment again. The shortest naturally formed fragments seen were seven segments long, but pieces three segments long regenerate perfectly. The head (of five segments) will not regenerate posteriorly unless at least three trunk segments are left attached to it. No cases of atypical or bipolar regenerations have been seen. In a few experiments with pieces from different levels of the anteroposterior axis, no differences in rate or amount of regeneration with respect to level could be detected; but regeneration is so rapid at room temperature that, for a proper study of the role of level in regeneration, regeneration rate would need to be slowed by manipulation of the temperature.

4. *Induction of fragmentation.*—In worms exceeding a length of 20 mm., fragmentation can be induced at will simply by removing the worms from the culture and placing them in dishes of fresh water without food. Since the worms are highly thigmotactic and restless when so isolated, it is desirable to provide them with something into which they can twine. For this purpose a coarse filamentous alga which the worms cannot eat has been kept on hand, and a small washed bit of this has been added to each isolated bunch of

worms. No doubt some thoroughly washed cotton would serve as well. Under such conditions at room temperature (25° C. or higher) most or all of the worms will fragment within 24 hours. Since fragmentation can be induced whenever desired, it becomes practical to experiment with the process of fragmentation itself, and it is to this problem that my attention has been devoted. Of the many possible problems for an attack on which *N. paraguayensis* offers highly suitable material, I have so far confined myself to two

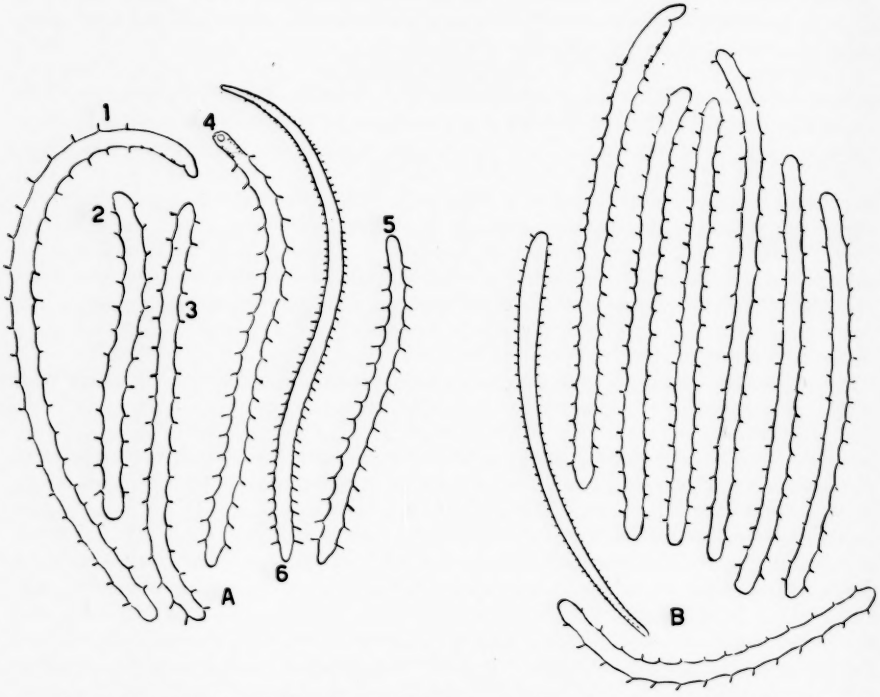


FIG. 1.—Fragments obtained from two worms. Worm *A* fragmented into six pieces, but not simultaneously, so that the levels of the fragments could be ascertained, except fragments 2 and 3, whose relative position is uncertain. The number of segments per fragment is: 1; 21 + 5 head segments; 2, 12; 3, 17; 4, 19; 5, 15; and 6, the tail piece, of over 50 segments. The actual lengths were: 7, 4, 5, 4, 4, and 6.5 mm., respectively. Worm *B* fragmented into eight pieces, the order of which could not be determined; the segment numbers are: 22 + 5, 22, 22, 18, 20, 19, 14, and tail piece of over 40 segments.

questions: What factors control the time of fragmentation; and can the number of fragments into which the worm breaks be experimentally altered?

THE ROLE OF EXTERNAL AND INTERNAL FACTORS IN THE TIME OF FRAGMENTATION

1. *Theoretical.*—Before proceeding to an account of the experiments, it seems desirable to indicate their theoretical basis. The lines of experiment followed are based upon the well-known conclusions of C. M. Child. In a long series of publications Child has

brought forward experimental evidence for the theory that "the organic individual is fundamentally a dynamic relation of dominance and subordination, associated with and resulting from the establishment of a metabolic gradient or gradients" (1915, p. 88). The region of highest rate of activity of the gradient becomes the apical end of the organism; and this end, by virtue of its high activity, controls and determines the developmental processes at other levels of the major axis. This control, however, has a distance limit, and beyond this limit physiological isolation occurs (Child, 1910, 1911*a*, 1911*b*, 1929), with consequent asexual reproduction in forms capable of regeneration. The range of dominance of the apical end depends on (1) the length of the animal, (2) the metabolic rate of the apical end relative to that of subordinate parts, (3) the efficiency of the conducting paths, and (4) the receptivity of the subordinate parts. Asexual reproduction can be controlled by altering experimentally any of these four factors. For example, incasing the growing-point of a plant or putting a cold block below the growing-point induces the sprouting of latent buds at lower levels of the axis (Child, 1919). According to Child's ideas, any factor which raises the metabolic activity of the head relative to other parts, or lowers the tendency of subordinate parts to become independent, or both, increases the range of dominance of the head and so should inhibit fragmentation in *N. paraguayensis;* and, conversely, any factor which diminishes the activity of the head relative to other parts or stimulates the subordinate parts will induce fragmentation. The following experiments furnish additional evidence for the truth of these generalizations.

2. *Procedure.*—As already indicated, the procedure is simple. Worms of mature length, 30–35 mm., except when the effect of size is under experimentation, are selected from the cultures, rinsed free from culture fluid, and placed in small, covered dishes of clean fresh water with a bit of filamentous alga. At first, Bear Mountain spring water was used for both cultures and experiments, but later the New York City tap water was found perfectly suitable and has since been employed after standing for a few days in a large receptacle. Ten to fifty or sixty worms were placed in each dish, which could then be subjected to any desired condition; or the water in the experimental dishes could be altered in any way wanted. Since fragmentation occurs very rapidly at room temperature, it was necessary in many types of experiments to slow down the process by lowering the temperature. The splendid temperature-regulating equipment of the Laboratory of Experimental Biology permits one to maintain any desired temperature for long periods of time. A temperature of 18° C. has been found most suitable for slowing the fragmentation process.

In counting the number of segments in the fragments, chloretone anesthesia has been employed. A few grains of chloretone are sprinkled on a Stender dish containing the fragments; and in a few minutes the latter become motionless, so that their segment number is easily determined. If the worms are not kept too long under chloretone, they are uninjured by the exposure.

The limitations of space do not permit a complete presentation of the numerous data accumulated. Preliminary experiments, experiments involving fewer than ten worms per dish, and the data on matters on which a negative or uncertain result was obtained have been omitted.

3. *The effect of size on fragmentation.*—It is a common observation that animals do not undergo fission or budding until they reach a certain size. In terms of Child's theories, asexual reproduction occurs when the posterior levels of the organism, because of in-

crease in length, get beyond the range of effective dominance of the head and so become physiologically isolated.

In *N. paraguayensis*, as in other lower invertebrates, the readiness to undergo asexual reproduction is related to size when other factors are constant. Worms of four different sizes were isolated, and the time of fragmentation recorded (Table 1). As readily seen from the table, the longest worms (30–35 mm.) fragment first, the majority within 1 day, the remainder within 2 days, after isolation. The worms of the next size category (20–25 mm.) fragment next; in some cases there is little, if any, difference observable in the time of fragmentation between them and the largest worms at high temperatures, such as 27° C. (Exper. 29, Table 1); but the difference becomes evident when the temperature is lowered (Exper. 53, Table 1). Worms 15–20 mm. long are noticeably slow to

TABLE 1

EFFECT OF SIZE ON THE TIME OF FRAGMENTATION AFTER ISOLATION

Numbers show the number of worms fragmented on day of observation

EXP. NO.	TEMP. (° C.)	NO. OF WORMS	DAYS	LENGTH OF WORM (MM.)			
				8–15	15–20	20–25	30–35
27	20	10	5	0	0	9	.....
			7	0	7	10	.....
53	22	15	1	0	0	2	11
			2	0	3	12	15
			3	0	8	13	.....
			6	8	14	15	.....
29	27	10	1	0	1	8	7
			2	0	3	10	9
54	27	15	1	0	4	3	10
			2	4	7	12	15
			3	4	12	14	.....
			6	10	12	15	.....

fragment, but most accomplish the act if given time enough. The smallest worms, 8–15 mm. long, often fail altogether to fragment; but the larger worms in this category may fragment after several days. Worms 8–10 mm. long were never observed to divide on isolation; such worms are, of course, in process of regulation to normal proportions.

The relation of size to fragmentation was so evident that relatively few experiments were performed on the matter.

Worms of 15-mm. length fragment into two pieces; those 20 mm. long, into three or four pieces; and the number of fragments increases with further growth in length. The actual length of fragments is, of course, shorter the smaller the worm; but it appears that the number of segments per fragment is not altered by size alone. However, this matter has not been fully investigated as yet. It is thought that a study of the segment number of fragments in relation to size will throw some light on the stage at which the fragmentation levels become fixed and the order of their fixation. Since the worms add on new segments only in front of the anal segment, it is evident that a study of the number of

segments in the head fragment, for instance, in worms of different sizes, will reveal whether the fragmentation level for the head fragment is fixed at an early age.

4. *The effect of temperature on the time of fragmentation after isolation.*—The time at which mature worms (30–35 mm.) will fragment after isolation in fresh water depends upon the temperature. At 25–30° C., 75–100 per cent of the worms will undergo fragmentation within 24 hours, and the remainder will fragment by the second day. At 22°–23° C., many—sometimes most—will fragment within 24 hours, the remainder by the second or third day after isolation. At 18°–19° C. some fragmentation occurs within 2 or 3 days, but the majority require 3 or more days. At 16°–17° C. fragmentation is much delayed. Generally no worms fragment before 3 or 4 days, and often only 50 per cent will have fragmented within 5 or 6 days. Frequently some worms remain intact for many days at such temperatures. At 15° C. the fragmentation is very slow, and ordinarily does not begin until 5 or 6 days after isolation. Usually the majority of the worms fail altogether to fragment under such conditions. At 13° C. fragmentation is altogether inhibited, and at temperatures lower than this death generally ensues in a few days. Worms which do not fragment within 5 or 6 days after isolation commonly fail to fragment altogether; a factor of starvation is probably involved here but has not been investigated.

As already mentioned, lowering the temperature constitutes a valuable means of slowing the fragmentation and so magnifying the differences in the action of conditions other than temperature.

5. *The effect of pH on the time of fragmentation.*—The lettuce cultures in which *Nais* is grown have, on reaching equilibrium, a pH of about 7.3. The Bear Mountain spring water, used at first for cultures and experiments, has a pH of 6.9 immediately after the bottles are opened, but rises to 7.8 on standing, presumably through escape of CO^2. The New York City tap water, which was adopted later for cultures and experiments has, on standing, a pH of 7.8. It became necessary to know whether small variations in pH were of any significance in fragmentation experiments and what, in general, is the action of pH on the time of fragmentation. Fully grown worms (30–35 mm.) were isolated into dishes of water having a range of pH from 4 to 9, obtained by adding appropriate amounts of acid or alkali to the water used. The pH was determined by comparison with Lamotte color standards. In each experiment from 17 to 30 worms were put into each pH, but the whole range mentioned was not covered in every experiment. The bunch of worms in each dish was rinsed two or three times with water of the desired pH, and the dish then filled nearly full and covered. To slow down the fragmentation process, all pH experiments were conducted at about 18° C. The dishes were examined after 2 or 3 days, and the number of worms fragmented in each determined by counting the unfragmented individuals. During such an experiment the pH in the experimental dishes alters; the acid water becomes less acid, the alkaline water less alkaline. Possibly a more precise result could be obtained by changing the water frequently during the experiment, but the results secured did not appear to justify more refined experimentation. The data, presented in Table 2, show that extremes of pH, namely, an acidity of 4 or an alkalinity greater than 8, delay the fragmentation, as compared with an intermediate pH. No definite repeatable differences were found in the range from 5 to 8.

In some previous experiments with acidity (Hyman, 1925) I found the carbonate content of the water to play a decisive role. In most of the experiments on pH with *Nais*, spring water was used. This has a much higher carbonate content than the New

York City tap water. However, comparisons of the two kinds of water with respect to the effect of pH on the fragmentation of *Nais* failed to show any difference.

6. *Action of potassium cyanide on the time of fragmentation.*—Potassium cyanide and other cyanide compounds are known to depress the oxygen consumption of the majority of animals. In planarians, for instance, the oxygen consumption can be depressed to 10 per cent of the normal by means of cyanide without damage to the animals (Hyman, 1919; Allen, 1919). The cyanides are, therefore, valuable agents for depressing or inhibiting a variety of phenomena. Since the fragmentation process in *Nais* must involve motor activity at the breaking levels, a depressing agent in proper concentration should

TABLE 2

ACTION OF pH ON THE TIME OF FRAGMENTATION AFTER ISOLATION

(Temperature, 18° C.)

Exp. No	No. of Worms	pH 4	5	6	7	8	8.5–9
		No. of Worms Fragmented					
82	20	12	.....	19	19	20	.....
83	18	.....	12	11	15	8	.....
84	20	.....	15	10	9	3	.....
85	20	4	7	6	13	0	0
86	21	.....	21	10	8	21	.....
87	23	15	23	21	16	22	.....
88	27	1	17	2	0	6	0
89	17	.....	5	8	9	.....	4
90	22	4	16	22	20	20	16
91	28	13	24	25	19	25	10
92	30	11	30	24	28	22	1
93	25	14	21	14	20	22	10
94	25	2	21	24	20	.....	.....
95	30	1	1	17	22	4	4

retard or inhibit fragmentation, by direct action on the breaking regions. The factor of indirect susceptibility may also be involved. According to Child (1913), regions of high metabolic rate, such as the head, are better able to adjust to the lower concentrations of depressing agents than are regions of lower rate. Nonlethal concentrations of cyanide may, therefore, act not only by directly suppressing the act of fragmentation but by increasing the dominance of the head through the greater ability of the head than the body to acclimate.

In a range of concentrations from 1/10,000 to 1/2,000,000 mol., potassium cyanide was found to retard markedly the fragmentation process of *Nais*, when the process is slowed down by suitable lowering of temperature (Table 3). The result is little correlated with the concentration of cyanide except that high concentrations, such as 1/10,000 cyanide (not presented in the table), altogether inhibit fragmentation within the time limits of the experiment; 1/25,000 mol. is also very inhibiting. The action of cyanide is presumably largely a direct one on the fragmentation process. The worms in cyanide do not differ in appearance or activity from the controls in water.

On theoretical grounds it might be possible to find some concentration of cyanide

which would inhibit the head more than the body (without injuring the worms), and hence accelerate fragmentation. In the early experiments with cyanide I thought I had obtained such a result, and so reported in an abstract (Hyman, 1935). Later, however, I was unable, after repeated trials, to verify the early result; and I must conclude that the increased fragmentation seen in several experiments in the cyanided worms, as compared with water controls, was accidental. These early, and more or less preliminary, experiments have been omitted from Table 3, as they could not be verified and as all the later and more extensive experiments gave an inhibiting action of cyanide. The range of concentration which would have an accelerating effect must be very limited; and since, as will be shown below, the physiological state of the worms is subject to much

TABLE 3

ACTION OF POTASSIUM CYANIDE ON THE TIME OF FRAGMENTATION

Temperature 18° C. Concentration of Cyanide in Terms of Molar Strength. Numbers show the number of worms fragmented at the time of observation (2 or 3 days after isolation). Last column gives fragmentation in water control.

Exp. No.	No. of Worms	.00004	.00002	.00001	.000005	.0000025	.000001	.0000005	Water Control
50	40	.....	20	23	.....	.....	.....	.....	40
61	20	1	8	7	.....	.....	.....	.....	11
67	22	4	9	6	.....	.....	.....	.....	18
68	23	.....	8	8	5	.....	.....	.....	22
69	23	.....	.....	12	12	16	.....	.....	21
74	60	3	8	.....	.....	.....	.....	.....	33
76	42	.....	9	13	.....	.....	.....	.....	38
77	60	.....	.....	.....	30	.....	.....	.....	53
78	40	.....	.....	.....	.....	6	.....	.....	35
80	40	.....	.....	.....	.....	25	19	12	37
81	60	.....	.....	.....	.....	9	22	29	59

variation, depending on cultural conditions, the effective concentration would probably vary with each lot of worms. These considerations have led me to believe that the experiment in question is impractical.

7. *Effect of decapitation on the time of fragmentation.*—Child has shown (1910, 1911*b*) that decapitation accelerates fission in planarians and induces fission at a smaller size than normally undergoes fission. The induction of fission by decapitation in planarians has been repeatedly verified in class experiments at the University of Chicago and has also been confirmed by Vandel (1922) for European species of planarians. It strongly supports the theory of asexual reproduction as the consequence of escape of posterior levels from the dominance of the head, since decapitation is the simplest method of eliminating the dominance of the head. *Nais paraguayensis* furnishes excellent material for the demonstration of the effect of decapitation on fission. The results are given in Table 4. At the higher temperatures, 25°–27° C., there is little, and sometimes no, difference in the percentage of fragmentation of normal and decapitated worms; but when fragmentation is slowed by lowering the temperature to 15°–22° C., then the accelerating action of decapitation on fragmentation is very evident.

8. *Decapitation in relation to temperature and size.*—Decapitation increases the number of worms which will fragment at lower temperatures, such as 15° C., and is a means of

inducing fragmentation at such temperatures when other temperature problems are being attacked. At 13° C., a temperature at which normal worms will not fragment at all, decapitated worms cast off the tail pieces; and sometimes the trunk may also fragment. The breaking-off of only the tail pieces at such temperatures may indicate that this region is the first to constrict off normally.

Decapitation also accelerates the fragmentation of small worms and may increase the percentage of fragmentation in the smaller sizes. However, decapitation will not induce fragmentation in worms of 10 mm. length.

9. *Behavior of halves and quarters.*—If worms are cut in half, the posterior halves fragment within 1 day at room temperature, while the anterior halves require 2–5 days

TABLE 4

EFFECT OF DECAPITATION ON THE TIME OF FRAGMENTATION

Last two columns give the number of worms fragmented in normal and decapitated lots.

Exp. No.	Temp. (° C.)	No. of Worms	Days	Normal	Decapitated
16	27	10	1	8	10
29	27	20	1	15	20
51	27	20	1	19	20
56	25	20	1	20	20
57	25	20	1	20	20
57	25	20	1	17	20
51	22	20	1	0	20
51	22	20	1	2	18
53	22	30	1	7	30
16	20	30	1	0	24
51	19	20	1	2	18
51	17	20	1	0	4
56	17	20	1	1	7
			2	8	16
			5	13	20
57	15	40	2	0	4
			4	20	32

for fragmentation. If worms are cut into anterior fourth and posterior three-fourths pieces, the latter fragment within a day, while the quarter-pieces fragment very slowly or not at all, beginning with the fifth day after cutting. In the case of anterior three-fourths and posterior quarter-pieces, the former fragment in 2 days, the latter in 1 day or not at all. These findings again show that the presence of the head inhibits fragmentation within a certain distance.

EXPERIMENTAL CONTROL OF FRAGMENT LENGTH

The problem here attacked is the question of controlling the number of pieces into which the worm fragments. The matter at issue is: Are the places at which *Nais* constricts into fragments predetermined or can they be altered by environmental means? The problem is evidently a statistical one in which large numbers of worms must be studied. While it would be possible to isolate individual worms and record the number of pieces into which they fragmented, mass experiments have seemed a more practical

method of attack. Fully grown worms (30–35 mm.) were allowed to fragment under whatever conditions were desired, and then the length of all the fragments obtained was recorded by counting their segments under chloretone anesthesia. It is true that the length of the segments varies somewhat along the axis, but only in the tail piece is there a marked reduction in segment length. The number of segments in the tail pieces cannot be accurately determined because the last segments are indefinite and not fully differentiated, but an estimate in round numbers of the number of segments in the tail pieces was at first kept. As no evidence was secured that the length of the tail pieces was correlated with any of the procedures tested, the tail pieces were disregarded in later experiments. The head pieces were recorded separately. The data then concern the number of segments in each fragment and are arranged in the tables to give the number of fragments of each segment length obtained in any given experiment. In counting the number of segments in the head pieces, the five head segments were disregarded.

No evidence was obtained of any relation between actual length of the fragments or number of segments per fragment and body level, except that, as already mentioned, the head fragment is always longer and contains more segments than any of the trunk fragments. The trunk fragments, so far as observed, are all of approximately the same length and segment number, without regard to level, and give a normal variation curve with respect to the number of segments. It is these variation curves and the displacements of their modes by experimental means which are the subject of the following experiments.

1. *Fragment length in relation to temperature.*—It seemed probable that fragment length could be altered by the temperature at which fragmentation occurred. Numerous experiments were set up in which lots of worms were placed for fragmentation in different temperatures, usually 25°–27°, 20°–22°, and 15°–16° C. The number of segments was counted in each of the hundreds of fragments obtained, and the results tabulated and studied. The result of all this labor was negative: the length of the fragments cannot be altered by the temperature at which fully grown worms fragment. This finding suggests that the levels at which fragmentation is to occur are already more or less fixed in mature animals.

At low temperatures, such as 15°–16° C., a considerable number of long pieces are obtained, as shown in Table 5. These pieces range from thirty to fifty or more segments in length, while body fragments typically do not exceed twenty-five segments in length. It is evident, however, that these long pieces do not represent a shift in fragment length by low temperature but are simply pieces which failed to carry out the final fragmentation. In short, low temperature partially suppresses the fragmentation process, so that breaking fails to occur at some of the predetermined levels. As already noted, many worms do not fragment at all at 15° C.

2. *Effect on fragment length of culturing worms at different temperatures.*—When placing worms to fragment at different temperatures failed to affect the fragment length, culturing worms at different temperatures seemed the next logical step. According to Child (1910, 1911*b*), culturing at low temperatures increases zooid length in planarians and *Stenostomum;* at high temperatures, decreases zooid length. The explanation given is that the slower growth rate at low temperature permits the dominant region to keep pace with the increase of length, and so extend its range of dominance and retard physiological isolation; while the rapid growth at higher temperature outruns the rate of extension of dominance and so permits earlier isolation of zooids. Van Cleave, how-

TABLE 5

COMPARISON OF FRAGMENT LENGTH AT 25°–27° AND 15°–16° C., SHOWING SUPPRESSION OF FRAGMENTATION AT THE LATTER TEMPERATURE

Numbers show the number of fragments of each length in each lot of worms. Length is expressed as number of segments per fragment.

No. of Segments	Exp. 16		Exp. 18		Exp. 19		Exp. 21		Exp. 24		Exp. 57	
	27°	16°	27°	15°	27°	15°	27°	16°	27°	16°	25°	15°
6								2				
7	2	1						1				
8								1				
9	1											
10		3	1					1				
11	2		2	1			1	1				
12	11	2	9	1			1	6				
13	9	1	19	2		2	13	6	3			1
14	8	7	30	2		1	26	6	6	7	3	
15	15	3	61	5	3	1	21	13	13	5	4	1
16	4	2	42	5	5	2	17	6	20	6	11	1
17	3	2	24	10	13	3	14	7	16	3	11	2
18	4	4	16	5	18	4	9	8	13	3	13	2
19	4		13	3	11	6	1	4	6	7	10	2
20			9	8	9	4	1	5	2	1	12	1
21		2	3	5	8	3	2	1	6	4	9	3
22		1	5	2	6	2		4	6		2	2
23			1	1	4	2		1	3	1	3	2
24		1	4	3		1						1
25				1							1	3
26				2				1		1		1
27				3		1				1		
28				2		2				1		3
29		1		2		3		1		6		
30		1		3		1		1		3		2
31						1		1		1		2
32		1		3		1						2
33				1		2		1		1		1
34						1				1		2
35				1		2				1		4
36				2		1				1		3
37				1		1				1		3
38				1		2						2
39				3								
40				1		1						
41												2
42												1
43		1		1								
44				2						1		
45												
46				1		1				1		
47						1						
48												1
49				1								1
50 or more				9		2						3

ever, has recently shown (1937) that in naids the contrary result is obtained with low temperature, namely, a shortening of zooid length. There is also considerable evidence in the literature (reviewed by Van Cleave, where references will be found) that in naids winter temperatures shorten zooid length and summer temperatures increase it. Van Cleave did, however, find that rapid growth at 20°–25° C. in good cultural and nutritional conditions results in short zooids, confirming Child on this point. Poor nutritional and cultural conditions increase zooid length.

The present experiments confirm the results of Van Cleave (although obtained independently) and extend them to another species of naid. Cultures made from new fragments were placed at 27° and 18°–20° C., respectively. Both cultures had ample food supply and were kept in optimal conditions, with frequent changes of water. When the worms had reached full size, lots were isolated from each temperature culture and allowed to fragment at both temperatures. As the worms grew much more rapidly in the higher temperature culture than in the lower, it was not always possible to compare the fragmentation from the two cultures simultaneously. This was done sometimes by letting the warm culture undergo a spontaneous fragmentation. The fragments from this fragmentation grew up to full length about the time the cold culture had first reached fragmentation size.

This experiment has been done in full twice; the main results are given in Table 6. In each experiment, lots of fully grown worms were selected at various times and set to fragment at both 27° and 18°–20° C. Not all of these trials are presented in the table; those omitted gave the same result. It is seen that the fragment length of either the warm or the cold culture remains the same regardless of the temperature in which the fully grown worms are placed for fragmentation, a result which confirms that reached in the preceding section, i.e., that temperature does not affect the fragment length of mature worms. On the other hand, the fragment length is consistently greater in the 27° worms than in the 18°–20° worms. Thus, when *N. paraguayensis* is grown at a relatively low temperature, the fragment length is shortened; and when it is grown at a relatively high temperature, the fragment length is increased, despite the fact that the worms obviously grow much more rapidly at the higher temperature and may pass through two natural fragmentations to one such in the colder culture.

Not only the trunk fragments but also the head fragments are shorter in the cold cultures and longer in the warm cultures. In Experiment 26 the head fragments in the cold culture ranged from eighteen to twenty-seven segments in length, with an average of 24.7, those of the warm culture, from twenty-eight to thirty-six segments in length, average 32.1. In Experiment 40 the head fragments of the cold culture varied from seventeen to thirty segments, average 22.7; of the warm culture, from twenty-four to thirty-eight segments in length, average 28.6.

It should be remarked that it has not been practical to experiment with lower temperatures because of the extremely slow growth at even 15° C.

3. *The effect of aging of the culture on fragment length.*—Throughout the experiments with temperature it was noticed that the average segment number of the fragments, although consistent within any one experiment, varied considerably in experiments carried on at different times, as will be seen in Table 6. At room temperature the average number of segments per fragment might be anything from fifteen to twenty-five. The source of these differences was puzzling for a long time until it was eventually noticed that fragment length bore some relation to the age of the cultures. Experiments were

then set up comparing fragment length in worms from fresh and stale cultures. The data, given in Table 7, show that the fragment length is consistently shorter in worms grown in freshly made cultures than in those grown in old cultures. A similar result is obtained by crowding the worms. New fragments were placed in fresh cultures—a small number of fragments in one, a large number in the other. The latter grew noticeably more slowly than the former. The fragment length of fully grown worms from the uncrowded culture is shorter than of similar worms from the crowded culture (Exp. 39,

TABLE 6

EFFECT OF CULTURING AT DIFFERENT TEMPERATURES ON THE LENGTH OF BODY FRAGMENTS

Numbers show the number of fragments of each length in the different temperatures

No. of Segments per Fragment	Exp. 26				Exp. 40			
	27° at 27°	27° at 20°	20° at 20°	20° at 27°	27° at 27°	27° at 18°	18° at 18°	18° at 27°
7				1				
11			1					
12					1	1		3
13				1	1	1	2	2
14			1	1	3	2	7	7
15			3	2	1	3	6	22
16	1	1	3	6	2	7	15	16
17	1	1	1	9	8	3	9	15
18	1 2		3	12	11	14	1	11
19	1 4			6	3	7	7	1
20	4 3	3	3	6	6	4	1	3
21	8 2	1	1	4	3	4		1
22	5 2	4			2	4		
23	8 2	2		2	3	3		1
24	4	4		1	2	5		
25	1 1	2			5	4		
26	2 2	2	1	1		2		
27	2 1				1	1		
28	1 2				1			
29	1	1						
30								
34			1					
35		1						
36				1				
37						1		
Av.	22.2	23.2	19.1	18.4	19.2	19.7	16.2	16.1

Table 6). The experiment with crowding was performed only once. In all of these experiments, also, the length of the head fragments follows that of the body fragments, being shorter in fresh and longer in aged cultures. These experiments with aging of the culture confirm the findings of Van Cleave (1937), who used another species, *Pristina longiseta*.

The experiments here recorded indicate that in *N. paraguayensis* the levels at which fragmentation will occur are already fixed in fully grown worms but that they can be altered by the conditions under which the fragments grow to maturity. Fresh culture medium and low temperature shorten the fragment length, or, in other words, increase

the number of fragments per worm; old culture medium and high temperature lengthen the fragments or decrease the number of fragments per worm. It seems probable that these two factors are really identical, that is, at low temperature the culture ages less rapidly than at high, and the effect is the same as culturing the worms in fresh medium.

TABLE 7

THE EFFECT OF AGING OF THE CULTURE AND THE NUMBER OF WORMS PER CULTURE ON FRAGMENT LENGTH

Numbers show the number of fragments of each segment length in samples from various cultures isolated for fragmentation simultaneously at room temperature.

No. of Segments	Exp. 35			Exp. 36		Exp. 41		Exp. 75			Exp. 39	
	Old	Old	New	Old	New	Old	New	Old	New	New	Crowd-ed	Un-crowd-ed
9		2			1		1					
10		1		1								
11								1				
12		1	2						1			
13	1	1	4		1		1		2	3		
14			6	2	4		5		9	5		4
15		1	17		5	1	6		11	10		5
16			13	1	8	2	13		11	11		12
17	1		3		13	6	19	1	8	9	4	8
18	6	1	11		17	3	25	4	3	4	1	13
19	3	1	8		9	2	6	2	3	3	10	3
20	18	2	5	3	8	7	8	3	1		12	5
21	9	3	3	1	9	5	7	1			7	2
22	3	2	4	1	5	2	11	4			6	
23	7	4		4	3	3	3	3	1		8	
24	3	8	1	2		7	3	3			3	
25	4	3	1	4		6		7				
26	3	1		3	1	10	1	3			1	
27	2	4		5	1	4		2				
28		3		2		4		2			1	
29		2		2		4		2				
30		1		4		3		1				
31												
32	1	1		2		2		1				
33			1									
34			1			1						
35				1								
36												
37												
38				1								
39				1								
Av.	21.4	22.6	17.3	25.5	18.3	23.6	18.2	23.3	15.9	15.9	20.8	17.1

The result is not explicable on the grounds of rate of growth. The worms do certainly grow more rapidly in fresh medium than in old, as can be seen by inspecting the posterior growing region. But the worms also grow much more rapidly at 27° than at 18°. The effect on fragment length of higher temperature is, however, the opposite of that of fresh medium, although both accelerate the growth rate. Similarly, both low tempera-

ture and old medium slow down the growth rate but have an opposite effect on fragment length.

The results are not easy to interpret. The available facts seem to indicate that in naids zooid length is controlled chiefly by the concentration of metabolic and putrefactive products in the medium during growth. It seems necessary to postulate a low degree of head dominance and a strong tendency for posterior regions to become physiologically isolated as zooids. When the concentration of metabolic and putrefactive products in the medium is low, the isolation of posterior levels as zooids occurs early and zooids are correspondingly short. A higher concentration of such products retards the tendency of posterior levels to isolate; zooid formation is consequently slower and the zooids longer.

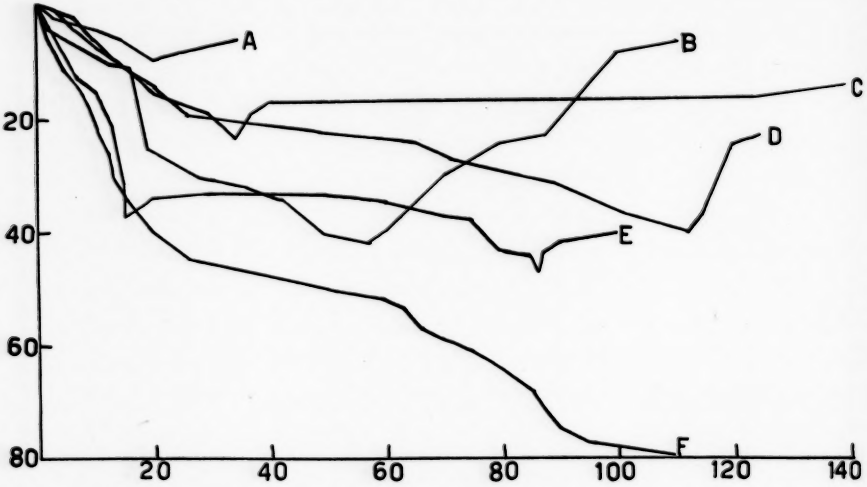


FIG. 2.—Graphs of the disintegration in cyanide of six worms of varying lengths. Segment number on the abscissa; time in minutes on the ordinate.

4. *Disintegration gradient of N. paraguayensis in cyanide.*—In the hope of obtaining some light on physiological conditions in *N. paraguayensis*, the disintegration of a number of individuals in 1/1,000 mol. potassium cyanide was watched and recorded as number of segments disintegrated against time. Some years ago I studied the disintegration gradients of oligochaetes (Hyman, 1916) and found that, in general, they are of the "double" type with disintegration proceeding from both ends toward the middle. The posteroanterior gradient of the posterior part of the body apparently results from the continuous growth of new segments there and seems to have little, if any, integrative value. In *N. paraguayensis* the disintegration gradient shows considerable individual variation, as illustrated in the graphs in Figure 2. Only in the smaller worms is the gradient often of the U-shape found in other oligochaetes (Fig. 2*A*), and some small worms have a continuously descending gradient with no posterior rise. These variations presumably depend upon the rate of growth of the posterior region. In the larger worms, disintegration always begins at the head and progresses posteriorly, giving a rather steep

gradient. This simple descending gradient, however, extends for only twenty to twenty-five segments. Posterior to this level the gradient more or less flattens out, and there is usually a long stretch in which segments disintegrate simultaneously or nearly so (Fig. 2*C*, *D*, *E*). This flattened part of the gradient may comprise the greater part of the worm but may be succeeded by a short posterior region of higher susceptibility (Fig. 2*D*, *E*). Sometimes the larger worms show a typical U-gradient (Fig. 2*B*), and sometimes they give a descending gradient following the flattened region (Fig. 2*F*). Not infrequently the posterior end fails to show any increased susceptibility; this presumably depends upon the rate of growth at the time of testing.

These results indicate that in this worm the head controls only a relatively short length of the axis and that most of the body of the adult animal is physiologically isolated. This isolation apparently begins at a relatively early stage, at which time the fragmentation levels become more or less fixed. No very definite indications of fragmentation levels show up in the disintegration gradients, although probably the sharp dips in the curves in *C*, *D*, and *E* represent such levels. It is also probable that the level where the descending anterior gradient passes into the flattened portion of the gradient is the place where the head fragment separates from the trunk.

SUMMARY

1. *Nais paraguayensis*, a South American fresh-water oligochaete of the family Naididae, reproduces asexually by fragmentation without the formation of typical fission planes. The worms grow to a length of 30–35 mm. and then spontaneously fragment into several pieces, usually six to eight. These pieces regenerate rapidly into complete normal worms.

2. Fragmentation can be induced within 24 hours by isolating fully grown worms into fresh water without food at a temperature of 25°–30° C.

3. The head fragment is typically longer than the body fragments, both absolutely and as to number of segments (typically twenty to thirty, exclusive of the five head segments). The body fragments are approximately equal in length and number of segments, typically ten to twenty. The tail fragment is of about the same length as the body fragments but contains many more segments (mostly thirty to fifty) because of the small size of the most posterior segments.

4. The time of fragmentation after isolation is dependent on size and is more delayed the smaller the worms. Worms 10 mm. or less in length do not fragment.

5. The time of fragmentation after isolation is dependent on temperature and is more delayed the lower the temperature. At 15° C. fragmentation is very slow, and many worms fail altogether to fragment. Fragmentation is usually completely inhibited at 13° C. or lower, and death generally ensues at 10° or lower.

6. Extremes of pH, 4 or below, and above 8 retard the fragmentation. No definite effect was seen in the range from pH 5 to 8.

7. All concentrations of potassium cyanide used (1/10,000 to 1/2,000,000 mol.) retard the fragmentation without affecting activity or appearance of the worms.

8. Decapitation accelerates the fragmentation in all sizes that will fragment and at all temperatures below 25° C.

9. The length of the fragments, that is, the places at which fragmentation occurs, are already fixed in mature worms and could not be altered by any procedures tried.

10. The length of the fragments from mature worms is the same regardless of the temperature at which fragmentation takes place; but at temperatures of 15°–16° C., long pieces may be obtained through suppression of some of the fragmentation levels.

11. The length of the fragments can be altered by the conditions under which fragments grow up to mature worms.

12. Worms cultured throughout their growth at lower temperatures (18°–20° C.) fragment into shorter pieces than those cultured at a higher temperature (25°–27° C.).

13. Worms grown in freshly made cultures fragment into shorter pieces than those grown in old cultures.

14. Worms grown in uncrowded cultures also fragment into shorter pieces than those from crowded cultures.

15. The results obtained under 12, 13, and 14 probably all depend upon one factor, the rate of accumulation of metabolic and putrefactive products in the culture during the growth of the worms. The higher the rate of accumulation of such products, the more slowly are the fragmentation levels established and the greater the distance between them, i.e., the longer the eventual fragments.

16. Disintegration in cyanide gives a simple anteroposterior disintegration gradient only at the anterior end. Over most of the body the gradient is irregular and very shallow, and long stretches of segments disintegrate simultaneously or nearly so. Some indications were seen of a differential susceptibility of fragmentation levels. The posteroanterior gradient typical of the posterior end of aquatic oligochaetes is weakly developed.

17. The results are interpretable on the basis of Child's theory of asexual reproduction as a physiological isolation from a dominant region.

LITERATURE CITED

AIYER, K. S. P. 1924. Reproduction in the aquatic oligochaete, *Nais paraguayensis* Mich. Ann. and Mag. Nat. Hist., Ser. 9, **14**:615.

ALLEN, G. D. 1919. Quantitative studies on the rate of respiratory metabolism of *Planaria*. I. The effect of potassium cyanide on the rate of oxygen consumption. Amer. Jour. Physiol., **48**:93.

CHILD, C. M. 1910. Physiological isolation of parts and fission in *Planaria*. Arch. f. Entw'mech., **30**:159.

———. 1911*a*. Die physiologische Isolation von Teilen des Organismus als Auslosungsfaktor der Bildung neuer Lebewesen und der Restitution. Vorträge und Aufsätze über Entw'mech. (ed. by W. Roux), **11**:157.

———. 1911*b*. Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. III. The formation of new zooids in *Planaria* and other forms. Jour. Exper. Zoöl., **11**:187.

———. 1913. Studies on the dynamics of morphogenesis, etc. V. The relation between resistance to depression agents and rate of metabolism in *Planaria* and its value as a method of investigation. *Ibid.*, **13**:103.

———. 1915. Individuality in organisms. Chicago: University of Chicago Press.

———. 1919. Physiological isolation by low temperatures in *Bryophyllum* and other plants. Science, **50**:362.

———. 1929. Physiological dominance and physiological isolation in development and reconstitution. Arch. f. Entw'mech., **117**:21.

HYMAN, L. H. 1916. An analysis of the process of regeneration in certain microdrilous oligochaetes. Jour. Exper., Zoöl., **20**:99.

———. 1919. On the action of certain substances on oxygen consumption. II. Action of potassium cyanide on *Planaria*. Amer. Jour. Physiol., **48**:340.

———. 1925. On the action, etc. VI. The action of acids. Biol. Bull., **49**:288.

———. 1935. Fragmentation in the naid *Nais paraguayensis* Mich., 1905. Abstract. Anat. Rec., **64**, Suppl. 1:79.

MICHAELSEN, W. 1905. Zur Kenntnis der Naididen. Zoologica, **44**:350.
STEPHENSON, J. 1930. The Oligochaeta. Oxford: Clarendon Press.
VAN CLEAVE, C. D. 1937. A study of the process of fission in the naid *Pristina longiseta*. Physiol. Zoöl., **10**:299.
VANDEL, A. 1922. Recherches expérimentales sur les modes de reproduction des planaires triclades paludicoles. Bull. biol. France et Belgique, **55**:344.

APPENDIX

THE CULTURE OF NAIS PARAGUAYENSIS

Shallow vessels are most convenient. Have 1–2 inches of water. Water should be of a type found suitable for other aquatic animals. Put lettuce leaves in water and bring them to a vigorous boil; but do not let boil, as this makes them too mushy. Thin, leafy types of lettuce are preferable, such as romaine and the kind known in New York City as Boston lettuce. Outer leaves discarded by grocery stores are suitable and usually may be had gratis. Place the boiled leaves in the cultures in the proportion of one fair-sized leaf to an 8- or 10-inch dish. Add worms immediately; do not let cultures stand without worms in them. Covering is desirable. Aeration is unnecessary. Temperatures of 25°–30° C. are best for rapid growth; temperatures below 20° should be avoided. As soon as the lettuce leaf is well occupied by worms or evidently going to pieces, add another leaf. Avoid too much lettuce. Disintegrated remains of old leaves should be constantly removed. The worms will commonly abandon old remnants and go on new leaves. Once or twice a week pour off most of the water and replace with fresh water. For best results entirely new cultures should be set up at frequent intervals. Too many worms in a culture inhibit growth; as soon as the worms become abundant, they should be subdivided into fresh cultures. Growth of algae in the culture is undesirable and can be eliminated by putting the cultures in total darkness for a few days. The presence of protozoans, crustaceans, rhabdocoels, etc., in the cultures should be avoided.

Nais paraguayensis is one of the most suitable invertebrates yet discovered for research and class purposes. It can be grown easily and in enormous numbers at negligible expense. It furnishes exceptional material for the study of growth, asexual reproduction, regeneration, action of physical factors and chemical composition of the medium, population biology, conditioning, etc. In addition, it constitutes excellent food for experimental fish and urodele larvae and is easier and more dependable to grow for such purposes than *Daphnia*.

Cultures will gladly be sent to interested zoölogists.

THE EFFECT OF PLANARIAN EXTRACTS AND EXUDATES UPON HEAD REGENERATION IN EUPLANARIA DOROTOCEPHALA

(One figure)

J. WILLIAM BUCHANAN
Department of Zoölogy, Northwestern University

THE establishment of the principle of organizers, which has been one of the important contributions to knowledge of the physiology of development of the last two decades, has been largely the result of work on vertebrate embryos, principally the amphibian embryo. As is well known, in the amphibian embryo intact living tissues, tissue extracts, tissues killed by heat, and other substances—for example, thyroxin—when applied under suitable conditions elicit responses that in some manner result in differentiations that are beyond the expected developmental fate of surrounding tissues. The literature on regeneration in the invertebrates contains evidence which shows that, with exceptions in the case of regeneration of the planarian head, only intact living tissues are capable of acting as organizers. In *Euplanaria dorotocephala* both living-tissue transplants and external chemical agents induce head formation, the former in the intact animal, the latter in regenerating fragments. This classical experimental animal therefore affords suitable material for further experimental analysis of the nature of inductive influences. The experiments about to be described are continuations and extensions of earlier work (Buchanan, 1922, 1923). They were planned to test the possibility that the anterior region of the planarian, *E. dorotocephala*, from which effective inducing transplants have been made (Santos, 1929) may contain or give off from the living tissue specific agents that induce head regeneration in pieces taken from posterior regions of the animal. The results appear to have an important bearing on the general problem of induction in form regulation and on the specific problem of the nature of the determining conditions in head regeneration in planarian pieces.

The application of the method of mass experiments to regeneration problems by Child (1911) has made it possible to examine with greater accuracy the conditions underlying reconstitution and form regulation. By this method it has been shown that many agents and conditions may cause the development of a head by a piece of a planarian that would otherwise undergo little or no regeneration. This evidence of induction of head formation is found in the greater frequency of occurrence of heads, in various degrees of completeness, among treated lots of pieces, as compared with their frequency in control lots of physiologically similar pieces taken from the same region of the animals and regenerating in water. Among the effective agents are the cyanides, various alcohols, anesthetics, alkaloids, inorganic acids and bases, organic acids, exposure to low temperature, and water conditioned by the animals (Child, 1911, 1912, 1916, 1920; Behre, 1918; Buchanan, 1922, 1923; Hinrichs, 1924; Rustia, 1925; Rulon, 1936, 1937).

Without exception, agents that are effective in increasing head frequency also cause decreases in head frequency when applied in suitable concentrations or intensities. Thus if pieces are treated immediately after section with weak concentrations or intensities, the number of heads or headlike anterior ends formed in a given lot of pieces is

significantly greater than among controls. But after treatment with higher concentrations or intensities, the head frequency is significantly less than among controls. The explanation of these remarkable results was first set forth by Child (1911); it is adopted by the writer in two previous papers (1922, 1923) and will be discussed farther on in the present communication.

Induction in planarian regeneration has also been demonstrated by the method of transplantation. If a fragment of living tissue is taken from the head region of a planarian and implanted posterior to the pharynx, the implant exhibits a distinct and positive inducing action, impelling the formation of a head which incorporates both adjacent and transplanted tissue (Santos, 1929). The action is not species specific, for transplants from *E. maculata* induce head formation in *E. dorotocephala*. But homoplastic transplants are effective only if the transplanted tissue is taken from an anterior region and placed in a posterior location.

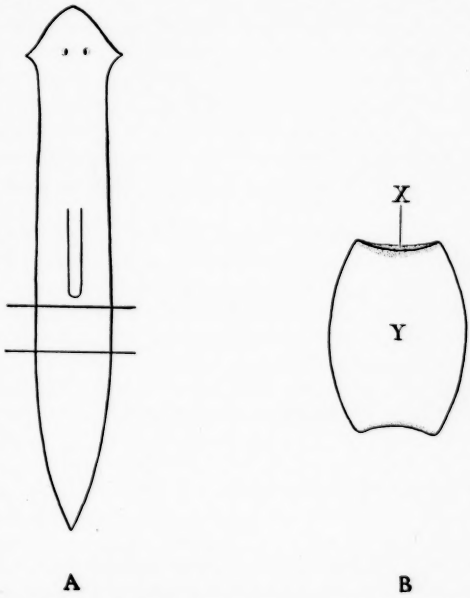


FIG. 1.—*A*, diagram of *E. dorotocephala*. Portion between transverse lines posterior to pharynx employed in experiments. *B*, diagram of an isolated piece designating the cells at and near the anterior cut surface as *X*, and the remainder of the piece as *Y*. *Y* also includes the posterior cut surface and adjacent stimulated cells.

MATERIALS AND METHODS

Abundant supplies of *E. dorotocephala* are available in this area. Collections are kept under conditions that are controlled with regard to food supply and temperature, and the selection of animals for experimentation was always made from stocks that had been so controlled for more than a week, usually 3 or 4 weeks after collection. Pieces approximately 1 mm. long were cut from the region just posterior to the pharynx from carefully selected animals of the same length (Fig. 1, *A*). In each experiment a number of such pieces, usually 50 or more, were set aside in water as a control. Similar lots were then cut and treated in various ways, as described farther on. After 10–12 days the number of heads and of other types of regeneration was determined for both control and experimental lots. Differences in the head frequency of the treated lots as compared with the control are attributed to the experimental treatment.

The method is attended by certain difficulties. Two control lots consisting of 50 pieces each, cut consecutively and as nearly as possible under identical conditions, do not always yield the same head frequency; the differences appear to be due to at least two uncontrollable elements: the operator cannot cut with the accuracy necessary to assure that all pieces are taken from exactly the same region of the animals, nor can pieces be

cut that do not vary somewhat in length. Moreover, there are undoubtedly distinct individual differences in the regenerative responses of the animals. The writer has repeatedly tested the method by comparing the head frequencies of lots of 50 cut consecutively, and of lots obtained by cutting 100 or more pieces and dividing at random into groups of 50 each. In no case did the range of difference in head frequency between comparable lots exceed 10 per cent. A similar analysis has recently been given by Rulon (1936). Consequently, in the data given below, differences between experimental and control lots exceeding 10 per cent are regarded as significant. Moreover, consistently recurring differences in the same direction of less than 10 per cent may also be regarded as indicative.

A second source of difficulty arises from the fact that whether or not a head will be regenerated by a piece is partially determined within a few hours after the piece is isolated. The cutting of 100 or more small fragments is a tedious and time-consuming task. By the time that the last pieces are cut, the appropriate distribution to flasks completed, and the experimental treatment applied, pieces first cut are several hours older than those last isolated.

In this particular work another difficulty developed because of the great number of animals required for many of the experiments. Although the form is plentiful and easily obtained, large stocks must be kept on hand because selections for experimentation must be made of animals of the same size. Consecutive day-to-day experiments could not be carried out because of lack of numbers of animals of uniform size. Stocks from which selections were made were therefore in the laboratory for different periods. It has been observed that after some weeks in the laboratory the head frequencies of controls increase. Thus repetitions desirable to test the validity of an observation could not always be made upon identical material.

In some American triclads the regulative reaction appears to be of an all-or-none type; either normal heads are regenerated or no regeneration occurs (Buchanan, 1933, 1936). In planarians regenerating fragments form all degrees of anterior regeneration from normal heads to complete absence of anterior tissue. This peculiarity in *E. dorotocephala* permits the detection of differences in the effect of an experimental agent to a finer degree than would otherwise be possible. For such a study it has been found convenient to establish categories of degree of head completeness among regenerating pieces (Child, 1911). Five classes of anterior ends have been distinguished: normal, in which the head is indistinguishable from the heads of collected animals; teratophthalmic, in which the head is normal in shape, or nearly so, but the eyespots are fused to a greater or lesser extent; teratomorphic, in which the head is a rounded protuberance with a single median eye; anophthalmic, in which the head is represented by some anterior outgrowth devoid of eyes; acephalic or headless, in which there is no anterior outgrowth; in this type of regenerate, posterior regeneration is quite complete but the anterior cut surface is simply contracted and filled with scar tissue. Agents which affect head frequency show their effects in several ways: (1) there may be a reduction in the number of regenerated anterior ends of all classes. This occurs when the agent is applied in relatively high concentrations or intensities. (2) There may be an increase in total number of regenerated anterior ends of all classes. Brief exposures usually yield this result. (3) The total number of regenerated anterior ends may not differ from that of the control, but the number in one or another class may be decreased or increased as compared with controls. To evaluate the effects of an agent it is necessary to consider not only the changes

in the total number of anterior ends regenerated but also differences between the various classes.

DATA

The most obvious approach to the problem of head induction by specific head-generated substances is to treat isolated pieces with extracts of head tissue. Consequently, in the first series of experiments pieces were taken from the region indicated in Figure 1, *A*, and treated with a simple water extract prepared as follows: heads were cut from 300 animals and placed in a mortar with a few drops of tap water. Grinding was carried on for 5 minutes. Water was then added to make 25 cc., the mass stirred vigorously and then allowed to settle. One hundred animals of approximately the same length were selected and the designated pieces cut. Fifty pieces selected at random for

TABLE 1

HEAD FREQUENCY AMONG REGENERATING PIECES OF *E. dorotocephala* AFTER TREATMENT WITH WATER EXTRACTS OF MASHED PLANARIAN HEADS

(Controls treated with tap water; in per cent)

Lot	No. of Pieces	Duration of Treatment (Hours)	Normal	Teratophthalmic	Teratomorphic	Anophthalmic	Headless	Dead
Control	75	16	17.3	56.0	0.0	9.4	14.6	2.6
Experimental	75	16	26.4	53.3	1.3	5.3	5.3	8.0
Control	50	17	70.0	20.0	0.0	4.0	6.0	0.0
Experimental	50	17	88.0	10.0	0.0	0.0	2.0	0.0
Control	50	14	52.0	24.0	0.0	18.0	6.0	0.0
Experimental	50	14	90.0	6.0	0.0	4.0	0.0	0.0

a control were placed in a beaker and covered with 25 cc. of tap water. The head extract was then filtered and poured over the remaining 50 pieces. Both lots were allowed to stand for periods up to 18 hours. The control lot was then removed and placed in 500 cc. of tap water. The experimental lot was washed in several changes of tap water and also placed in 500 cc. of tap water. Water over the pieces was changed every other day. After 10 or more days the regenerates were examined. Three such experiments were carried out, with the results shown in Table 1. An inspection of the data demonstrates that pieces subjected to the head extract regenerated more heads than did the controls.

Attempts were then made to prepare ether and acetone extracts of planarian heads, but the results were not fruitful and the method was abandoned. It was found that when heads were mashed and shaken with ether, no visible deposit was left when the ether evaporated. In fact, when 300 heads were thus treated with 3 cc. of ether, the evaporating ether left no oily stain on white filter paper. Acetone extracts were so minute as to be quite impractical for use.

The data in Table 1 do not answer the question as to whether the increased number of heads regenerated in the experimental lots is due to a specific inductor or to an alteration of the physiological conditions in the isolated pieces, thereby setting up a system conducive to head regeneration. The specific inductive capacity of the head extract

was tested as follows: 10 animals, 16 mm. long, were beheaded and individually placed in a Petri dish on ice. After they had become quiet a glass needle, prepared by drawing out a capillary tube, was used to inject a small quantity of head extract into each animal in the region from which the regenerates in Table 1 had been taken. Ten control animals were injured in the same region by the same needle, but without injection. After 11 days each animal was examined under the binocular microscope. In both experimental and control animals several had undergone fission. In the others the injuries had healed without any trace of outgrowths of any sort.

The procedure was varied in another experiment. Ten animals were injured in the region just posterior to the pharynx and then placed in 10 cc. of head extract for 18

TABLE 2

HEAD FREQUENCY AMONG REGENERATING PIECES OF *E. dorotocephala* IN RELATION TO VOLUME OF WATER IN CONTAINERS

(In per cent; 50 in each lot)

Lot	Water Volume (Cc.)	Duration of Treatment	Normal	Tera-tophthal-mic	Terato-morphic	Anoph-thalmic	Head-less	Dead
Control	500.0	12 days	36	38	0	20	6	0
Experimental	0.2	18 hr.	50	32	2	14	2	0
Control	500.0	14 days	26	50	0	16	8	0
Experimental	0.2	16 hr.	22	40	4	20	10	4
Control	500.0	10 days	18	32	0	36	14	0
Experimental	0.2	17 hr.	18	36	2	30	14	0
Control	500.0	11 days	12	20	2	46	20	0
Experimental	0.2	14 hr.	6	28	2	50	14	0
Control	500.0	12 days	16	32	2	12	34	4
Experimental	1.0	20 hr.	16	46	4	16	18	0

hours. Controls similarly injured were placed in the same quantity of water. The results were entirely negative; there was no evidence that the head extract had in any way altered the course of wound-healing or induced any changes whatever in the site of injury.

Many years ago Child (1912) showed that when pieces of *E. dorotocephala* were allowed to regenerate in water in which whole animals had been kept, head frequency was appreciably higher compared with controls regenerating in fresh water. This suggests that substances excreted by the intact animal act on head regeneration in the same direction as do extracts of tissues. In the next series of experiments an attempt was made to determine whether or not such exudates are derived solely from the head. Some data were also obtained showing a relation between water volume and head frequency. In Table 2 it appears that keeping the pieces in 0.2 cc. water for periods up to 20 hours after section has no appreciable effect upon head frequency when compared with pieces regenerating for the full period in 10 cc. of water. But in Table 3 it is shown that if heads or posterior ends are added to a 10 cc.-water volume, together with a regenerating piece,

a crowding effect occurs. In two cases head frequency is increased and in two others it is decreased by the presence of additional tissue.

Ten experiments on crowding in a small water volume are given in Table 4. Eight show increases in head frequency whether the crowding is due to the addition of heads, tails, or other pieces from the same region of the animal.

From Tables 2, 3, and 4 it appears that (1) short treatment of pieces in exceedingly small volumes of water has no effect upon head regeneration; (2) crowding pieces with either head, tail, or other tissue in small volumes of water for a period up to 24 hours induces increases in head frequency in most cases but may also cause decreases; (3) if crowded in larger volumes of water for the duration of the regenerating period, there is

TABLE 3

HEAD FREQUENCY AMONG REGENERATING PIECES OF *E. dorotocephala* IN RELATION TO WATER VOLUME AND IN THE PRESENCE OF HEADS AND POSTERIOR ENDS

(In per cent)

Lot	No.	Water Volume (Cc.)	Conditions	Normal	Tera-tophthal-mic	Tera-to-morphic	Anoph-thalmic	Head-less	Dead
Control	50	10	1 piece	44.0	18.0	0	22.0	16.0	0.0
Experimental	50	10	1 piece + 3 heads	54.0	26.0	0	12.0	8.0	0.0
Control	50	10	1 piece	62.0	22.0	2	4.0	10.0	0.0
Experimental	50	10	1 piece + 3 tails	78.0	10.0	0	2.0	2.0	8.0
Control	50	4	1 piece	14.0	34.0	0	18.0	38.0	0.0
Experimental	50	4	1 piece + 1 head	8.0	32.0	4	20.0	32.0	4.0
Control	80	6	1 piece	3.7	16.2	0	35.0	38.7	0.0
Experimental	80	6	1 piece + 2 heads	0.0	20.0	0	33.7	43.7	2.5

a tendency for head frequency to increase; (4) exudates of heads, tails, or of the pieces themselves induce changes in head frequency.

It is then clear that head regeneration may be influenced by the proximity of living planarian tissue if the quantity of water forming the environment is sufficiently small. It is also shown that the controlling influence is derived from living tissue from several regions of the animal. Further evidence of the nature of this influence was then sought. The experiments so far carried out are quite inadequate to determine whether or not planarian tissue may be the source of emanations that influence head frequency; but so far as they go, it is clear that under the conditions of this work the action of living tissue in influencing head regeneration will not operate through glass or quartz $\frac{1}{2}$ mm. in thickness. In each of these experiments 100 pieces were cut as before. As a control, 50 were placed in a pyrex glass beaker with 100 cc. of tap water. The beaker was set in a larger beaker and the space between the beaker walls filled with water. From 150 to 300 freshly cut heads were placed in the water in the outer beaker, resting in the water jacket approximately 5 mm. thick between the two beakers. The remaining 50 pieces were ar-

TABLE 4

HEAD FREQUENCY AMONG REGENERATING PIECES OF *E. dorotocephala* AFTER EXPOSURE TO FRESHLY CUT PLANARIAN HEADS, POSTERIOR ENDS, AND WHEN TWO PIECES FROM THE SAME REGION ARE PRESENT

(Each piece was placed in 0.2 cc. water, and in experimental lots the head or other portion added. After treatment all pieces were removed to five hundred cc. flasks, fifty to each flask. Forty or fifty pieces in each lot.)

Lot	Treatment	Duration (Hr.)	Normal	Teratophthalmic	Teratomorphic	Anophthalmic	Headless	Dead
Control	Water	24	40.0	48.5	0	5.7	5.7	0
Experimental	With heads	24	58.8	38.2	0	3.0	0.0	0
Experimental	With tails	24	51.5	42.4	0	6.0	0.0	0
Control	Water	14	6.0	28.0	2	50.0	14.0	0
Experimental	Two pieces	14	8.0	33.0	2	46.0	11.0	0
Control	Water	20	16.0	46.0	4	16.0	18.0	0
Experimental	With heads	20	14.0	34.0	2	18.0	30.0	2
Control	Water	18	32.0	30.0	0	20.0	16.0	2
Experimental	With heads	18	54.0	24.0	2	6.0	10.0	4
Control	Water	24	12.0	48.0	0	20.0	16.0	4
Experimental	With heads	24	28.0	28.0	8	8.0	12.0	16
Control	Water	24	36.0	32.0	0	12.0	16.0	4
Experimental	With heads	24	28.0	56.0	0	4.0	12.0	0
Control	Water	17	18.0	36.0	2	30.0	14.0	0
Experimental	With heads	17	14.0	28.0	2	38.0	16.0	2
Control	Water	17	20.0	36.0	0	26.0	14.0	4
Experimental	With heads	17	32.0	28.0	2	24.0	14.0	0
Control	Water	18	50.0	32.0	2	14.0	2.0	0
Experimental	With heads	18	24.0	40.0	0	20.0	16.0	0

TABLE 5

HEAD FREQUENCY AMONG REGENERATING PIECES OF *E. dorotocephala* WHEN SEPARATED FROM PLANARIAN HEADS BY GLASS AND QUARTZ WALLS ½ MM. THICK

(In per cent)

Lot	Normal	Teratophthalmic	Teratomorphic	Anophthalmic	Headless	Dead
Glass (75)	6.6	30.6	5.3	36.6	20	1.3
Quartz (75)	5.2	36.6	2.6	33.3	16	6.6
Glass	14.0	34.0	0.0	20.0	32	0.0
Quartz	14.0	30.0	0.0	18.0	38	0.0

ranged in an identical setup, except that the inner beaker containing the regenerates was made of quartz. The water in all beakers was changed daily.

The experiment was modified as follows: 50 heads were placed in a beaker containing 200 cc. of water and freshly cut heads added. Fifty other pieces were placed in a small quartz beaker containing 100 cc. of water which was then set in a larger beaker containing 100 cc. of water and a like number of heads, 150–300. The results of two of these experiments are given in Table 5. They show no significant differences between the various lots of regenerates. The conclusion is reached that, while head frequency may be increased by adding heads to water containing regenerating pieces, separating the heads and the regenerates by glass or quartz ½ mm. thick prevents the effect of the heads on regeneration.

DISCUSSION

It is shown by the data that tissue extracts, including perhaps products of autolysis, and excretory products of living planarian tissue when applied under suitable conditions cause a given lot of planarian pieces to regenerate a higher percentage of heads or head-like anterior ends than do control lots not so treated. Similar increases in head frequency have been recorded after treatment with cyanides, anesthetics, alkaloids, acids, bases, low oxygen tension, low temperature, conditioned water, and mechanical stimulation (Child, 1911, 1912, 1916, 1920; Buchanan, 1922, 1923; Hinrichs, 1924; Behre, 1918; Rustia, 1925; Rulon, 1936, 1937).

It can hardly be supposed that this extensive group of agents and conditions act upon the pieces in a specific way or confer head-forming properties on cells that otherwise lack such powers. In the first place, the effective agents represent such a wide range of physical and chemical properties that the possibility that all contain a single component which causes heads to develop is precluded. In the second place, it has been shown for most of these agents that they may affect head frequency in either direction, depending on concentration or duration of treatment. One is therefore forced to the conclusion that in inducing head regeneration all these agents act upon some system or condition inherent in the piece, altering such system quantitatively rather than qualitatively.

An interpretation which fits the facts was advanced many years ago. According to Child (1911, and other papers, including Child and Watanabe, 1935), whether or not a new head is regenerated in a piece of *E. dorotocephala* depends upon the relative intensities of the metabolic processes, or certain of them, of two regions of the isolated piece, *X* and *Y* in Figure 1. If the activity of the *X* cells is high in relation to that of the *Y* region, a new head forms. If the activity of the *Y* region is high in relation to that of the head-forming *X* cells, the head-regenerating properties of the *X* cells are more or less inhibited and the new anterior end falls within the categories of more or less complete heads described above and figured in numerous papers by Child, and also by the writer and others.

The importance of the inhibitory action of the *Y* region has recently been emphasized by Child (Child and Watanabe, 1935). It was shown that the sectioning of the nerve cords is an effective factor in determining the inhibitory activity of the *Y* region. But inhibition of head formation by the *X* cells cannot wholly be ascribed to the stimulation of the nerve cords by section, for it has been shown by the writer (Buchanan, 1922, 1923) that those narcotics which act primarily upon nervous tissue are highly effective in increasing head frequency when applied to certain pieces immediately after section, but

are quite ineffective when applied 48 hours later. On the other hand, general protoplasmic depressants such as KCN are quite effective in increasing head frequency when applied at either time.

Proofs of the parallelism between physiological activity of the *X* and *Y* regions of the piece and the production of new heads have been established by studies of carbon dioxide output, of oxygen consumption, and of susceptibility to toxic agents (Child, 1914; Robbins and Child, 1920; Hyman, 1919; Buchanan, 1922, 1923). It has been shown that depressants such as the common anesthetics inhibit the metabolic activity of both the *X* and the *Y* regions (Buchanan, 1922). If the *X* cells are more profoundly affected than the *Y* region, then head regeneration is inhibited, as shown by the fact that the head frequency of a given lot of pieces so treated is less than that of a control lot. If the metabolic activity of the *Y* region is more inhibited than that of the *X* cells, a situation is created which makes for increased independence of the *X* cells. Under such conditions in a given lot of pieces the head frequency is greater than in control lots.

The most probable interpretation of the effects on head regeneration of extracts of planarian tissue and of exudates from the intact animal is that such substances are depressants and that their effects on head determination are not different in principle from those of other depressants.

Not much is known concerning the nature of the products of planarian metabolism or of the contents of macerated and autolyzed planarian tissue. Child (1930) showed that *E. dorotocephala* conditions a hypotonic medium, for 10 animals do not disintegrate as rapidly in 125 cc. of distilled water as do 10 like animals in 250 cc. This was confirmed by the writer (Buchanan, 1930), who suggested that the exudates must form osmotically active substances after excretion. But Allee (1933) failed to confirm this suggestion, for he found that water conditioned by *E. dorotocephala* has a slightly lower osmotic pressure than control water taken from the same well. Oesting and Allee (1935) showed that *E. novangliae* excretes minute quantities of calcium into a hypotonic water, and the writer (1933) found that extremely low concentrations of calcium protect the organism against cytolysis by hypotonic agents. But it is extremely doubtful if such minute quantities of calcium induce the states of depression necessary to cause changes in head frequency or that it accounts for the effects shown here. Hyman (1932) showed that the exudates of living planarians do not combine with iodin to any marked extent, but this fact gives little intimation of their physiological effects.

Changes in the water in which *Euplanaria* are living include an increase in carbon dioxide content and a decrease in dissolved oxygen. It was shown by Rulon (1936) that carbon dioxide in suitable concentrations increases the head frequency of regenerating pieces. The depressive effect of insufficient oxygen upon metabolism is too well known to merit comment. Thus either of these two conditions may be called upon to account for the head-frequency increases observed in lots that had been crowded or were in small quantities of water. The fact that plugging the glass capsules caused death after 24 hours is further evidence of the injurious action of interference with gas exchange. But in the case of experiments with filtrates of mashed heads, some other substances must be responsible.

The results reported and reviewed here do not admit of interpretations based upon the specific action of some inductive or organizing substance. This is of considerable importance, for the work of Holtfreter (1933), Waddington, Needham, and Needham (1933), Waddington (1933), Fisher *et al.* (1935), and others on the nature of induction in

vertebrate embryos has encouraged the view that organizers exercise control over the development of adjacent tissues by means of specific substances secreted by or diffusing from the controlling elements. According to Holtfreter (1933) the amphibian organizer retains its potency as an inducing agent after a variety of severe treatments, including heating up to 120°. Tissues which show no inducing capacity while living acquire the property when boiled. Fisher and his co-workers (1935) conclude from their work that the effective inducing agent is a nucleo-protein. Apparently the inductive capacity is not species-specific (Holtfreter, 1933; Waddington, 1933; see the discussion by Brensted, 1937).

The diversity of treatment accorded the organizer without destroying its effectiveness, the range of tissues and of tissue extracts that are effective, and numerous other facts call the concept of organizer specificity into question. When the evidence is considered together with the evidence from regeneration and transplantation studies in invertebrates, the facts suggest that the embryo and the regenerate are activable systems which may be influenced quantitatively by a wide variety of agents. At least such conditions appear to be responsible for the experimental alteration of the developmental fate of the *X* cells in regenerating pieces of *E. dorotocephala*, as shown by the evidence in the present communication and in other papers on experimental alteration of head frequency by Child and his students.

SUMMARY

Water extracts of freshly cut planarian heads induce increases in the number of heads regenerated by isolated cross-pieces of *E. dorotocephala* when such extracts are applied for periods less than 24 hours immediately after the sections have been cut.

Head extracts when introduced into the intact animal in a posterior region, or when applied to animals injured in a posterior region, do not induce head formation at the site of injury.

If living heads or posterior ends are included with regenerating pieces in small volumes of water, 0.2 cc., for less than twenty-four hours after cutting, the regenerates show the effects of crowding. In some cases head frequency is increased and in others it is decreased by such treatments.

If the crowding is less intense but continues throughout the regeneration period there is a tendency for head frequency to increase.

Evidence of an effect on head frequency of living planarian heads separated from the regenerates by glass or quartz walls was entirely negative.

These results when considered together with the fact that many other agents and conditions alter head frequency, e.g., low temperature, organic and inorganic acids, bases, anesthetics, and alkaloids, support the conclusion that whether or not a head regenerates in a piece of *E. dorotocephala* depends upon quantitative physiological conditions within the piece; specific action of particular agents cannot be called upon to explain the experimental results.

LITERATURE CITED

ALLEE, W. C. 1933. Studies in animal aggregations. Further analysis of the protective value of biologically conditioned fresh water for the marine turbellarian, *Procerodes*. Physiol. Zoöl., **6**:1–21.

BEHRE, E. H. 1918. An experimental study of acclimation to temperature in *Planaria dorotocephala*. Biol. Bull., **35**:277–317.

BRENSTED, H. V. 1937. Organisatorstoffe. Protoplasma, **28**:123–30.

BUCHANAN, J. W. 1922. The control of head formation in *Planaria* by means of anesthetics. Jour. Exper. Zoöl., **36**:1–47.
———. 1923. On the nature of the determining factors in regeneration. *Ibid.*, **37**:395–416.
———. 1930. The nature of disintegration gradients. I. The significance of a gradient in susceptibility to distilled water in *Planaria*. *Ibid.*, **57**:307–29.
———. 1933. Regeneration in *Phagocata gracilis* (Leidy). Physiol. Zoöl., **6**:185–204.
———. 1935. An analysis of physiological states responsible for antero-posterior disintegration in *Planaria dorotocephala*. Protoplasma, **22**:497–512.
———. 1936. Notes on an American cave flatworm, *Sphalloplana percaeca* (Packard). Ecology, **17**:194–211.
CHILD, C. M. 1911. Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. I. The axial gradient in *Planaria dorotocephala* as a limiting factor in regulation. Jour. Exper. Zoöl., **10**:265–320.
———. 1912. Studies, etc. IV. Certain dynamic factors in regulatory morphogenesis of *Planaria dorotocephala* in relation to the axial gradient. *Ibid.*, **13**:103–52.
———. 1914. Studies, etc. VII. The stimulation of pieces by section in *Planaria dorotocephala*. *Ibid.*, **16**:413–41.
———. 1916. Studies, etc. IX. The control of head form and head frequency in *Planaria* by means of potassium cyanide. *Ibid.*, **21**:101–25.
———. 1920. Studies, etc. X. Head frequency in *Planaria dorotocephala* in relation to age, nutrition, and motor activity. *Ibid.*, **30**:403–18.
———. 1930. The susceptibility of *Planaria* to potassium cyanide in relation to hydrion concentration and to certain changes in salt content of the medium. Physiol. Zoöl., **3**:90–134.
CHILD, C. M., and WATANABE, Y. 1935. The head frequency gradient in *Euplanaria dorotocephala*. Physiol. Zoöl., **8**:1–40.
FISHER, F. G., WEHMEIER, E., LEHMANN, H., JÜHLING, L., and KULTSCH, K. 1935. Zur Kenntnis der Induktionsmittel in der Embryonal-Entwicklung. Ber. Deutsche Chem. Ges., **68**:1196–99.
HINRICHS, M. A. 1924. A study of the physiological effects of caffein upon *Planaria dorotocephala*. Jour. Exper. Zoöl., **40**:271–300.
HOLTFRETER, J. 1933. Der Einfluss thermischer, mechanischer, und chemischer Eingriffe auf die Induzierfähigkeit von Triton-Keimteilen. Arch. f. Entw'mech., **132**:225–306.
HYMAN, L. H. 1919. Physiological studies on *Planaria*. II. Oxygen consumption in relation to regeneration. Amer. Jour. Physiol., **50**:67–81.
———. 1932. The axial respiratory gradient: Experimental and critical. Physiol. Zoöl., **5**:566–92.
OESTING, R. B., and ALLEE, W. C. 1935. Further analysis of the protective value of biologically conditioned fresh water for the marine turbellarian *Procerodes wheatlandi*. IV. The effect of calcium. Biol. Bull., **68**:314–27.
ROBBINS, H. L., and CHILD, C. M. 1920. Carbon dioxide production in relation to regeneration in *Planaria dorotocephala*. Biol. Bull., **38**:103–22.
RULON, O. 1936. The effects of carbon dioxide, the hydrogen ion, calcium, and experimental conditioning on reconstitution in *Euplanaria dorotocephala*. Physiol. Zoöl., **9**:170–203.
———. 1937. The effects of certain organic acids on reconstitution in *Euplanaria dorotocephala*. *Ibid.*, **10**:180–96.
RUSTIA, C. P. 1925. The control of biaxial development in the reconstitution of pieces of *Planaria*. Jour. Exper. Zoöl., **42**:111–42.
SANTOS, F. V. 1929. Studies on transplantation in *Planaria*. Biol. Bull., **57**:188–97.
WADDINGTON, C. H. 1933. Induction by coagulated organizers in the chick embryo. Nature, **131**:275–76.
WADDINGTON, C. H., NEEDHAM, J., and NEEDHAM, D. M. 1933. Beobachtungen über die physikalisch-chemische Natur des Organisators. Naturwiss., **21**:771–72.

THE MICROSCOPIC ANATOMY OF TWINS AND DOUBLE MONSTERS OF FUNDULUS HETEROCLITUS

MARIE A. HINRICHS
Department of Physiology and Health Education, Southern Illinois Normal University, Carbondale, Illinois

IN A previous series of experimental studies on the modification of development of *Fundulus heteroclitus* by means of ultraviolet radiation, it was possible to produce a considerable number of embryos showing varying degrees of axial duplication (Hinrichs and Genther, 1931).[1]

METHODS

The experimental procedure may be briefly reviewed as follows: fertilized eggs of *F. heteroclitus* were exposed in a shallow layer of sea water for a period of 1 minute, at intervals varying from 5 to 116 minutes after fertilization, to the action of unscreened radiation from a Cooper-Hewitt quartz-mercury-vapor arc, at a distance of 11.5 cm. from the center of the arc. The period most favorable for twin production seemed to be within the 30 minutes just before the appearance of the first cleavage furrow.

For descriptions and sketches of the types found among the 179 experimentally produced twins and double monsters the reader is referred to our earlier report (Hinrichs and Genther, 1931).

It became evident from a study of the gross specimens that it was not always possible to estimate the degree of duplication of organs or parts thereof. Accordingly, the material was sectioned serially for microscopic study.

DATA

Eighty-two sectioned specimens were studied, all of which showed some degree of axial duplication, varying from a complete permanent separation of axes, with subsequent independent development thereof, to somewhat irregular forms with bodies wider than normal, but with little or no obvious external indication of duplication.

Those twins or double monsters which resulted from exposures made as early as from 10 to 15 minutes after fertilization most frequently showed a pronounced effect in the brain region, where there seemed to be a cellular overgrowth with little organization. (Such malformed brains have also been produced in chick embryos [Hinrichs, 1927].) In such embryos the eyes were poorly developed also, sometimes amounting only to a concentration of pigment in the areas where eyes should normally appear. More posteriorly, such a nervous system may become more normal in outline, yet show definite duplication of the neural canal, usually a ventral bifurcation. Others, while obviously greatly irregular in structure—some appearing almost solid with convolutions and masses of rapidly proliferating cells—could still be found to have two distinct divisions each with its own distorted canal—in many instances the mirror image of its mate. Still

[1] These studies covered a period of four summers at Woods Hole, Mass., and were completed in 1930. The material thus obtained was prepared for microscopic study by Dr. Ida Genther under a grant from the Radiation Fund of the National Research Council.

others appeared wider than normal and were found to have an appended mass of brain tissue more or less irregular in outline and structure, which when followed posteriorly seemed to become part of a partially double neural tube. In such instance, when the duplication appeared ventrally, there also appeared below each half of the cord a complete notochord which was more or less vacuolated and usually wider than a single normal notochord.

Usually the heart and gut and its derivatives were normal in their arrangement, as were other organs. In some instances, however, where the ventral duplication of the neural tube was marked and the notochords parallel, the latter were separated by a mass of muscle tissue which lay just ventral to the neural tube. Such embryos showed a midline depression on the surface.

Embryos of this type have been traced and sketched at intervals throughout development, beginning with the first appearance of any axial delimitation, at which time two parallel equipotential axes could be demonstrated very close to each other.

Another interesting type of twin was formed as a result of the simultaneous development of two equipotential normal antipodean axes. Members of such pairs remained distinct from each other throughout, and on microscopic study showed complete mirror-imaging of organs.

Where the angle between the axes of the developing embryos became less than 180°, the chances for remaining separate entities became less the nearer these axes approached each other on the germ ring, and the chances for subsequent fusion appeared greater. One embryo usually gained advantage over the other, and as development continued the smaller component became differentially more modified. In other words, in this series there was a gradation of inhibitions of the smaller components including embryos differentially inhibited in the midline, so that eyes appeared closer together than normally, or even fused. Sometimes the inhibiting action was great enough to cause the brain to appear irregular and little organized, with an apparent cellular overgrowth, eyes reduced to mere pigment spots, and sooner or later the component fused with the larger more normal component. This is the true "autosite-parasite" type of double monster. In some of these embryos there seemed to be an abnormally large amount of gut tissue.

In some instances the only obvious duplication appearing externally was a large optic cup with double lenses on one side. Microscopically, such embryos showed a malformed brain and posteriorly a duplication of the neural canal, especially in the ventral region. Sometimes such a duplication extended only partway posteriorly, while the rest of the embryo seemed normal, microscopically as well as grossly.

DISCUSSION

From a study of the microscopic examination of serial sections of experimentally produced twins and double monsters, it appears that the means employed in this experiment—namely, ultra-violet radiation—is particularly suited because there is no subsequent dilution factor to be reckoned with as is the case in experiments with chemicals, and the latent effect seems relatively short. Therefore it is possible to interrupt the course of normal development at a given period for only a relatively short interval, and in this way produce a developmental modification of those embryonic parts which at the time are most susceptible to change. Such a period of differential susceptibility has been found immediately to precede the subsequent development of an organ. This suggests

an explanation for a condition as described above, in which development is interfered with only in the parts of the embryo developing early, when exposure to radiation is made within the first 15 minutes after fertilization.

The optimum time for interfering with normal axial development seems to come shortly before the two-cell stage in embryonic cell division.

SUMMARY

A microscopic study of serial sections of artificially produced twins and double monsters of *F. heteroclitus* presents new evidence for the following:

1. Completely separated twins and partially fused double monsters which develop equipotentially show a definite mirror image of organs. This seems to be true whether or not the embryos are normal in size and development, and also whether they lie 180° apart on the germ ring or close to each other and parallel, with partial fusion.

2. When one embryo develops more rapidly than another with which it is anatomically joined, it appears to do so at the expense of the smaller embryo. The latter shows a differential inhibition of developing organs. In all such cases there is a definite union, sometimes slight, of the two embryos by means of the nervous system. Also the two axes must be potentially different at the outset.

3. There may be some degree of duplication present—e.g., in the central nervous system—which can be detected only microscopically.

4. Duplication may be present in some parts of the developing embryo and be absent in others, e.g., the heart and gut may be single while parts of the nervous, muscular, and skeletal systems show evidence of duplication.

By this study added evidence is presented:

5. Ultra-violet radiation is an effective means of modifying embryonic development.

6. The effects produced are differential in character, i.e., those parts of the embryo which at the time of exposure are physiologically the most active, are the ones to be modified in development.

LITERATURE CITED

HINRICHS, M. A., 1927. Modification of development on the basis of differential susceptibility to radiation. IV. Chick embryos and ultraviolet radiation. Jour. Exper. Zoöl., **74**:309–42.

HINRICHS, M. A., and GENTHER, I. T., 1931. Ultraviolet radiation and the production of twins and double monsters. Physiol. Zoöl., **4**:461–85.

THE INFLUENCE OF THYROXIN ON EYE FORMATION IN PHAGOCATA GRACILIS (HALDEMAN)

(Six figures)

BERNAL R. WEIMER, CHARLES W. PHILLIPS, AND DAGNEY M. ANDERSEN
Bethany College, West Virginia

THE study of the endocrine glands and the physiological effects of their secretions has claimed the attention of investigators for a number of years. The literature in the field of vertebrate endocrinology is voluminous. Moreover, there is gradually accumulating data relating to the effect of vertebrate endocrine secretions on the invertebrates. At present this literature reveals many diverse and apparently contradictory conclusions. Nowikoff (1908) reported an acceleration of the division rate of *Paramecium* when thyroid substance was added to cultures of these animals. A similar effect was reported for *Paramecium* by Budington and Harvey (1915). Shumway (1917) reported that thyroid substance increased the division rate of *Paramecium aurelia* or *P. caudatum* almost to 65 per cent. Abderhalden and Schiffmann (1922) also reported an increase in division rate of *Paramecium* as a result of thyroid extract. Cori (1923) found that thyroxin powder (Parke, Davis & Co.) in alkaline solution accelerated the division rate of *P. putrinum*, but that thyroxin produced no effect. Burge and Estes (1925) found that insulin added to cultures increased sugar metabolism in *Paramecium*, but that thyroxin had no effect. Large amounts of thyroxin apparently were toxic. Woodruff and Swingle (1922), as a result of their studies, concluded that "all evidence from studies on *Paramecium* to the effect that thyroid products accelerate cell anabolism is erroneous." Torrey (1923) and Torrey, Riddle, and Brodie (1924) reported that thyroxin depresses the division rate of *Paramecium*. However, the latter authors report an increase in metabolism as measured by the increase in rate of contraction of the contractile vacuoles.

Some experimental work is reported on the effect of endocrines on arthropods. Hanko (1912) found that *Asellus communis* regenerated missing structures twice as fast in an extract of the hypophysis. Lecithin and glycogen also hasten regeneration in a measure. Kunkel (1918) fed the larvae of *Lucilia* on mammalian thyroid and found a slight retardation of growth of the larvae, and consequently the size of the resulting pupae was smaller. However, animals fed on thyroid tend to pupate more rapidly and have shorter periods of pupation. Romeis and von Dobkiewicz (1921) found that *Calliphora* larvae fed on thyroid develop more slowly than the control animals, and that there was little or no morphological difference between the thyroid-fed animals and the control. Kahn (1921) could find no change in time of metamorphosis of the larvae of *Corethra*, *Ecdyurus*, and *Tenebrio* when fed on desiccated thyroid. Von Herwerden (1923) found a decrease in time of onset of sexual maturity of *Daphnia pulex* when fed on desiccated adrenal cortex. Extract from glands of pregnant animals seemed most effective. Terao and Wakamori (1924) reported that thyroid material increased the number of eggs laid by the second generation of silkworms. Kopćc (1926) found little or no effect induced by the administration of thyroid material to the caterpillar of *Lymantria*, except a diminution in weight.

Ashbel (1935) reported an increase in the respiration of invertebrate tissue when treated with thyroid extract. This increase ranges from 23 per cent in the fresh tissue to an increase of 300 per cent in the desiccated tissue. Synthetic thyroxin, however, had no effect on oxygen consumption. Wulzen (1916) found that planarians fed on pituitary gland showed an increase in fission rate. He also reported that the thyroid has a positive influence on the rate of division and growth of *Planaria maculata*.

There is a wide divergence of opinion among investigators as to the role of metabolic rates in amphibian metamorphosis. There has existed a widespread general assumption that metamorphosis was accompanied by a rise in metabolism (Jahrisch, 1920; Uhlenhuth, 1921; Huxley, 1925; Schulze, 1930, and others). In fact, a rise in metabolism with accompanying metamorphosis has been reported by the investigators mentioned above. However, Etkin (1934), from his experiments and after a critical review of the literature, concludes that "there is no reason at present for ascribing any causative significance in metamorphosis to the supposed metabolism-accelerating influence of the thyroid."

Opinion seems to be divided, however, whether in experimental metamorphosis an agent, such as thyroxin is responsible for an increase in metabolism, or whether metamorphosis itself brings about the increase. Nevertheless, irrespective of the metabolic relations resulting from the treatment of the amphibia with the thyroid extracts or crystalline thyroxin, it has been definitely shown that these substances do hasten amphibian metamorphosis.

This paper presents the results of a series of experiments which show the effect of solutions of thyroxin upon an invertebrate, the common fresh-water flatworm, *Phagocata gracilis* (Haldeman). These experiments are intended to be merely preliminary and to serve as an introduction to other experiments which are now in progress or planned to be carried out in this laboratory in the future.

PROBLEM

It has long been known by various investigators that the turbellarians have remarkable powers of regeneration. Child and others have compiled a wealth of experimental data with respect to regeneration in *Euplanaria dorotocephala*. The metabolic relations existing in or induced by various agents in cut pieces of *E. dorotocephala* and their effect on regeneration have been further investigated and discussed by Buchanan (1922, 1923). It has been shown that the frequency of head formation in *E. dorotocephala* is dependent upon both the level at which the sections are made and the length of the sections. Further, it has been found that when a section is cut from the animal certain changes in the rate of the metabolic activity of the cut pieces occur. Various reasons are assigned for this increased rate of metabolic activity. It has been shown that cells near the cut surface are stimulated by the act of section. This must be regarded in part as due to wound stimulation. It has been shown also that not only are the cells of the cut surface stimulated by the act of section, but the entire piece is thus affected. This stimulation is usually temporary, subsiding in less than forty-eight hours. It is necessary to emphasize here that not only does wound stimulation affect these cells, but all the cells in the piece are isolated and freed from the dominance of the more anterior regions. Isolation of parts or groups of cells in many organisms brings about rapid differentiation and the initiation of a new organism. It is generally agreed that these processes involve a change in the rate of matabolism.

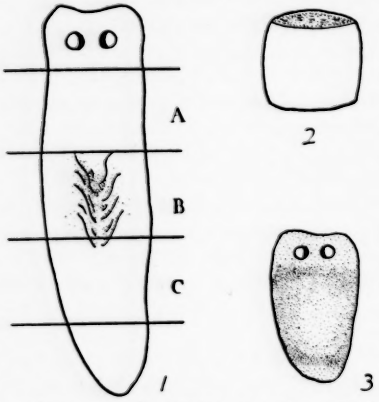
Experimental evidence has shown that in a piece of an organism undergoing recon-

stitution there often prevails a high rate of metabolism. Weimer, in unpublished research, has found that reconstitution can be retarded in *Pelmatohydra oligactis* Pallas (Schulze) by lowering the metabolic rate by use of low temperatures.

It was with these considerations in mind that the following preliminary experiments were designed. It was deemed advisable and more accurate to base all observations on the rate of reconstitution of some specific structure rather than on a semigeneralized structure such as the entire anterior end of an animal. For ease and accuracy of observation all data in the following experiments relate to the rate of eye formation.

METHOD

The animals used in these experiments were procured from springs and streams near Bethany. These stock animals were kept in the laboratory in water obtained from the streams in which they were collected. During this period they were not fed. After three days the largest animals of as nearly the same size as possible were selected from this stock. They were placed in culture water in the bottom of inverted finger bowls, and sectioned in the following manner:



FIGS. 1–3.—(1) Sketch of entire animal showing the method of cutting and the designation of levels; (2) appearance of typical cut section; (3) appearance of regenerated piece 10 to 12 days after sectioning.

First, the head region just back of the eyes was cut off and discarded. The main body region of each animal was then sectioned into three equal parts or levels exclusive of the most posterior body region which was discarded. Thus, each piece had two cut surfaces (see Figs. 1–3). The most anterior piece was designated as *A*, the middle piece as *B*, and the posterior piece as *C*.

The *A*, *B*, *C* regions were placed, as soon as cut, in separate finger bowls containing the desired solutions. Usually ten or fifteen animals were used for each series in the experimental solution, and the same number of animals as used in the experimental series was placed in culture water to serve as controls.

The thyroxin solution was made up as follows: 10 mg. of thyroxin (E. R. Squibb & Son) was placed in 1,000 cc. of water taken directly from the water in which the stock animal was living. This solution was shaken and stirred at various intervals. According to Kendall (1929), thyroxin is only slightly soluble in water. At no time have we attempted to analyze the amount of thyroxin present in the solution. After sectioning, the finger bowls containing the control animals and the animals in thyroxin solution were placed on a water table with a temperature of approximately 24° C. The animals were not in direct light.

After a lapse of 10 days, observations were made as to the number of pieces with eyes and the number dead. Observations were made on alternate days until all the animals in the thyroxin solution had developed eyes. In the tabulation of the data it seemed more convenient from the standpoint of comparison to show the results of the observations by percentages rather than by number.

Each time the experiment was repeated, fresh animals, fresh solutions, and the same methods were used. The results of these experiments are summarized in Tables 1, 2, and 3 and in Graphs 1, 2, and 3.

RESULTS

Before discussing the data summarized in the tables, attention is called to the fact that a somewhat similar series of experiments had been made in this laboratory twelve years ago by E. Morton Miller and Irving Tittler. These experiments were of an exploratory nature and were not run in sufficient numbers and with the same uniform

TABLE 1

RATE OF EYE FORMATION IN PIECES FROM *A* LEVEL

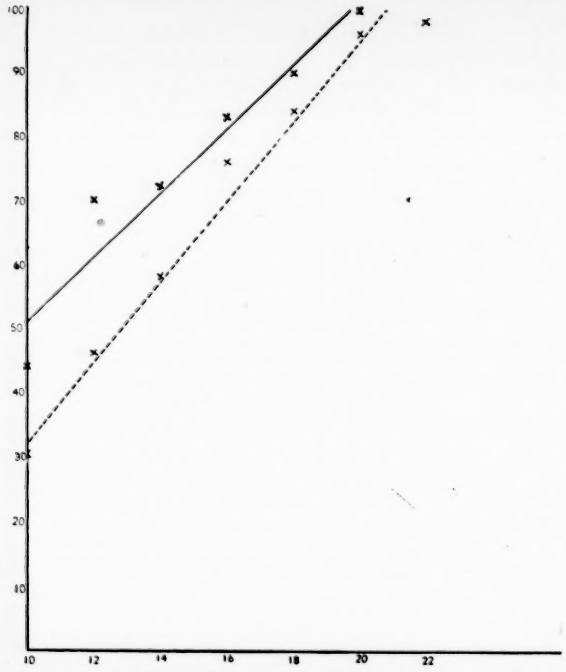
Observation	No. Animals	Thyroxin	Control	Percentage of Increase of Thyroxin over Control
1 (10th day)	50	22—eyes 44 per cent	15—eyes 30 per cent	14
2 (12th day)	50	35—eyes 70 per cent	23—eyes 46 per cent	24
3 (14th day)	50	36—eyes 72 per cent	29—eyes 58 per cent	14
4 (16th day)	50	42—eyes 84 per cent	38—eyes 76 per cent	8
5 (18th day)	50	45—eyes 90 per cent	42—eyes 84 per cent	6
6 (20th day)	50	50—eyes 100 per cent	48—eyes 96 per cent	4
7 (22d day)	50	.....	49—eyes 98 per cent	2

technique throughout as that used in the present experiments. However, the results of those experiments agreed essentially with the data presented here.

The study of Table 1 which summarizes the data for the series of experiments on the *A* sections shows that the greatest increase in eye formation of the pieces in thyroxin solution as compared to the controls is found on the tenth, twelfth, and fourteenth day after sectioning. These results are also shown in Graph 1. After that time both the control pieces and the pieces in the thyroxin solution regenerate at about the same rate, although the pieces in thyroxin solution show a differential of 4–8 per cent over the controls.

A study of the data summarized in Table 2 and shown in Graph 2 for the *B* pieces, shows practically the same results as for the *A* pieces except that the pieces in the thyroxin solution show a relative increase in percentage over the control in the later part of the experiment.

The data for *C* level, summarized in Table 3 and depicted in Graph 3, show that there

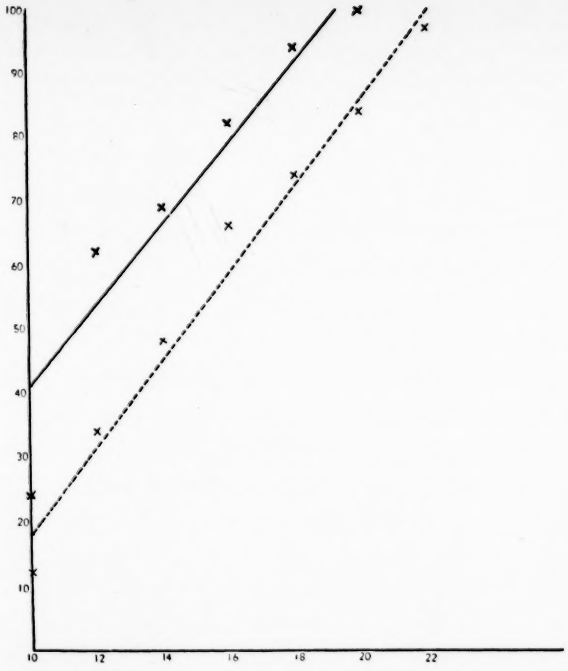


GRAPH 1.—Graph showing the difference in rate of eye formation between pieces from the *A* level in thyroxin solution and those in ordinary water. The ordinates represent the rate of eye formation expressed in percentage and the abscissas represent the time interval in days after sectioning when observations were made. The solid line —— shows the percentage of eye frequency in pieces in thyroxin solution, and the broken line - - - - - - shows the frequency of eye formation in pieces in culture water (control).

TABLE 2

RATE OF EYE FORMATION IN PIECES FROM *B* LEVEL

Observation	No. Animals	Thyroxin	Control	Percentage of Increase of Thyroxin over Control
1 (10th day).....	50	12—eyes 24 per cent	6—eyes 12 per cent	12
2 (12th day).....	50	31—eyes 62 per cent	17—eyes 34 per cent	28
3 (14th day).....	50	37—eyes 74 per cent	24—eyes 48 per cent	26
4 (16th day).....	50	41—eyes 82 per cent	33—eyes 66 per cent	16
5 (18th day).....	50	47—eyes 94 per cent	37—eyes 74 per cent	20
6 (20th day).....	50	50—eyes 100 per cent	42—eyes 84 per cent	16
7 (22d day).....	50	.....	46—eyes 92 per cent	8



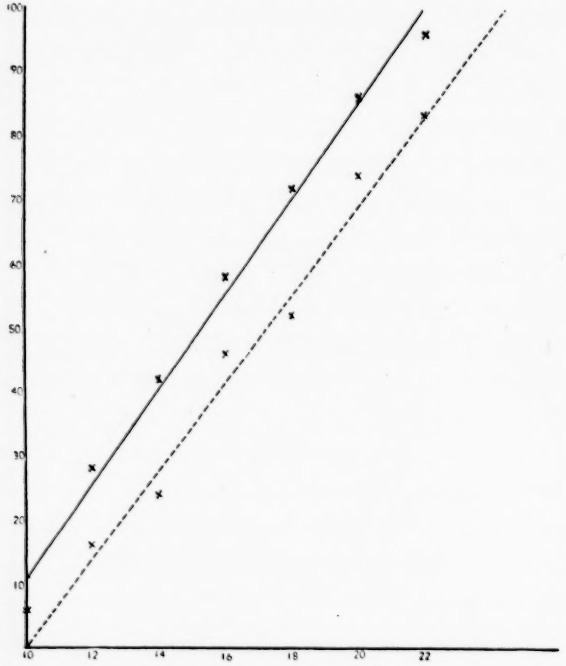
GRAPH 2.—Graph showing the difference in rate of eye formation between pieces from the *B* level in thyroxin solution and those in ordinary water. The ordinates represent the rate of eye formation expressed in percentage and the abscissas represent the time interval in days after sectioning when observations were made. The solid line —— shows the percentage of eye frequency in pieces in thyroxin solution, and the broken line - - - - - - shows the frequency of eye formation in pieces in culture water (control).

TABLE 3

RATE OF EYE FORMATION IN PIECES FROM *C* LEVEL

Observation	No. Animals	Thyroxin	Control	Percentage of Increase of Thyroxin over Control
1 (10th day)	50	3—eyes 6 per cent		6
2 (12th day)	50	14—eyes 28 per cent	8—eyes 16 per cent	12
3 (14th day)	50	21—eyes 42 per cent	12—eyes 24 per cent	18
4 (16th day)	50	29—eyes 58 per cent	23—eyes 46 per cent	12
5 (18th day)	50	36—eyes 72 per cent	26—eyes 52 per cent	20
6 (20th day)	50	43—eyes 86 per cent	36—eyes 72 per cent	12
7 (22d day)	50	48—eyes 96 per cent	39—eyes 78 per cent	18

is a percentage increase of the pieces in thyroxin as compared to those of the control. We also find, however, that conditions differ somewhat in this level from the behavior shown for the pieces at the *A* and *B* levels. Thus, the pieces of the *C* level begin to show the greatest percentage differences about the sixteenth day after sectioning. This difference between experimental pieces and controls continues throughout the experiment. The fact that the pieces from the anterior levels regenerate more rapidly than the pieces from the *C* level, in both thyroxin and controls, is in accordance with most observations



GRAPH 3.—Graph showing the difference in rate of eye formation between pieces from the *C* level in thyroxin solution and those in ordinary water. The ordinates represent the rate of eye formation expressed in percentage and the abscissas represent the time interval in days after sectioning when observations were made. The solid line —— shows the percentage of eye frequency in pieces in thyroxin solution, and the broken line - - - - - - shows the frequency of eye formation in pieces in culture water (control).

with respect to regeneration in all *Planaria*, namely, that the rate of regeneration is slower the more posterior the piece. Some exceptions to this rule are noticed in those *Planaria* which have a subordinate zooid present which eventually separates by fission. However, in *P. gracilis* there is no evidence at present to indicate the existence of a second zooid. Further, if a second zooid were present in the *C* level, one would expect an increase in rate of regeneration for these pieces over that shown in *B*.

The summary of the total data just presented for all the series is generally true of the

various individual experimental series which were run. There were some slight differences between the percentages of the series, but these might be ascribed to such factors as slight differences in the concentration of the thyroxin solutions; fluctuations in temperature; physiological differences of the animals; and other factors. The differences, however, are so slight that the results for the individual series coincide remarkably with those that are summarized in the tables for the total series.

DISCUSSION

The results of the data just presented show quite clearly that pieces of animals placed in a solution of crystalline thyroxin in water will regenerate and reconstitute more rapidly than pieces of animals kept in culture water under the same conditions. A large amount of work done by various investigators indicates quite clearly that thyroxin does increase the rate of metamorphosis in certain vertebrates. Also it has been shown that the rate of metamorphosis in amphibians can be increased by various other compounds, particularly iodin compounds (see summary in Kendall, 1929).

Opinion seems to differ as to the reaction of thyroxin and the iodin compounds as affecting metamorphosis. One view holds that metamorphosis is due to a change in the rate of metabolism of the tissues. Swingle, Helff, and Zwemer (1924) concluded that the increase in metabolism and the hastened onset of amphibian metamorphosis were due to the chemical reactions induced by the thyroxin molecule. Garisch concluded that thyroid brought about its effect through its influence on metabolism. Helff (1926) has found a progressive increase in oxygen consumption for metamorphosing larvae of *R. pipiens*. Kendall (1929), in summarizing a number of other experiments, reports as follows:

> From the results which have been obtained, it cannot be assumed that the metamorphosis is due primarily to a change in the basal metabolism. Neither is it clearly shown that there is an increase in the basal metabolic rate in all forms of amphibia after metamorphosis.

Kendall (1929) further suggests that the influence of thyroxin on the rate of metamorphosis is not due directly to its influence on the rate of energy production.

So far as these experiments are concerned, no attempt has been made to determine whether or not there was an increase in the rate of metabolism. In a letter Hyman suggested that thyroxin here may act as a depressant, which permits the regenerating part to become independent and the regenerating process to proceed more rapidly. The fact that thyroxin was apparently more effective on the *C* pieces would seem to be a confirmatory indication that the rate of regeneration is a result of depression rather than stimulation. With this in mind we have planned further experiments for the very near future. Nor do these experiments exclude the possibility that some slight impurity of the thyroxin may be responsible for the change observed in these experiments. One fact worthy of consideration is that, so far as eye formation is concerned, both the pieces in thyroxin and those in control apparently began this phase of the process of reconstitution after a lapse of approximately the same number of days. However, the rate at which eye formation progresses—once this process has begun—is much higher for those pieces in thyroxin than for those of the controls. This might suggest that, as in amphibian metamorphosis, two processes may be concerned here. Further, the fact must be kept in mind that there may be a wide divergence in the physiological process involved in the metamorphosis of amphibian larvae and the reconstitution of pieces from a flatworm.

SUMMARY

The anterior head region just back of the eyes and the most posterior region of the flatworm *P. gracilis* (Haldeman) were removed, and the remaining body sections were divided in three levels, the most anterior designated as *A*, the middle as *B*, and the posterior as *C*. Some of the pieces, segregated according to levels, were placed in culture water and others in thyroxin solution. Pieces from the *A* level in thyroxin solution showed an increased rate of eye formation in percentage ranging from 2 to 24 per cent (mean value approximately 10 per cent) over those in the control; from the *B* level, an increased rate of 8–28 per cent (mean value approximately 18 per cent) over the control; from the *C* level, an increased rate of 6–20 per cent (mean value approximately 14 per cent) over the control. Thyroxin solution apparently accelerates the rate of eye formation in re-constituting pieces of *P. gracilis*.

LITERATURE CITED

ABDERHALDEN, E., and SCHIFFMANN, O. 1922. Weiter Untersuchung über die von einzelnen Organen hervorgebrachten Substanzen mit spezifischer Wirkung. VII. Chemotaklische Versuche an Paramecien und Untersuchungen über die Geschwindigkeit ihrer Teilung unter den Einfluss von Optonen aus verschiedenen Organen. Arch. ges. Physiol., **94**:206.

ASHBEL, R., 1935. Action of thyroid extract on the respiration of tissues of invertebrates. Nature, **135**:343.

BUCHANAN, J. W. 1922. The control of head formation in *Planaria* by means of anesthetics. Jour. Exper. Zoöl., **36**:1.

———. 1923. On the nature of the determining factors in regeneration. *Ibid.*, **37**:395.

BUDINGTON, R. H., and HARVEY, H. F. 1915. Division rate in ciliate Protozoa as influenced by thyroid constituents. Biol. Bull., **28**:304.

BURGE, W. E., and ESTES, A. M. 1925. The effect of insulin, thyroxin and temperature on the sugar metabolism of *Paramecium*. Jour. Metabol. Res., **7–8**:183.

CHILD, C. M. 1924. Physiological foundations of behavior. Chicago.

CORI, G. T. 1923. The influence of thyroid extracts and thyroxin on the rate of multiplication of *Paramecia*. Amer. Jour. Physiol., **65**:295.

ETKIN, W. 1934. The phenomena of anuran metamorphosis. II. E. Oxygen consumption during normal metamorphosis. Jour. Physiol. Zoöl., **7**.

HANKO, B. 1912. Über den Einfluss einiger Lösungen auf die Hautung, Regeneration und das Wachstum von *Asellus communis*. Arch. f. Entw'mech., **34**:477.

HELFF, O. M. 1926. Studies on amphibian metamorphosis. II. The oxygen consumption of tadpoles undergoing precocious metamorphosis following treatment with thyroid and di-iodotyrosine. Jour. Exper. Zoöl., **65**:69.

HERWERDEN, M. A. VON. 1923. Der Einfluss von kleinen Quantitaten Nebennierenrinde des Rindes auf das Wachstum der Süsswasserschnecke Limaea. Arch. f. mikr. Anat. u. Entw'mech., **98**:505.

———. 1923. Der Einfluss der Nebennierenrinde auf das Wachstum und die Fruchtbarkeit von *Daphnia pulex*. *Ibid.*, p. 220.

HUXLEY, J. 1925. Studies on amphibian metamorphosis II. Proc. Roy. Soc., B, **98**:113.

JÄRISCH, A. 1920. Über die Wirkung der Schilddruse auf Kaulquappen. Pflügers Arch., **179**:159.

KAHN, R. H. 1921. Über Schilddrüsenfütterung an Wirbellosen. Arch. ges. Physiol., **192**:86.

KENDALL, E. C. 1929. Thyroxine. New York.

KOPĆC, S. 1926. Is the insect metamorphosis influenced by thyroid feeding? Biol. Bull., **50**:339.

KUNKEL, B. W. 1918. The effects of the ductless glands on the development of the flesh flies. Jour. Exper. Zoöl., **26**:255.

NOWIKOFF, M. 1908. Über die Wirkung des Schilddrüsenextraktes und einiger anderer Organstoffe auf Ciliaten. Arch. f. Protistenk., **11**.

OLIVER, G. O., and SCHEAFFER, E. A. 1895. On the physiological action of extract of pituitary body and certain other glandular organs. Jour. Physiol., **18**.

ROMEIS, B., and DOBKIEWICZ, L. VON. 1921. Experimentelle Untersuchungen über die Wirkung von

Wirbeltierhormonen auf Wirbellose. I. Der Einfluss von Schilddrüsenfütterung auf Entwicklung und Wachstum der Schmeissfliege (*Calliphora vomitoria*). Arch. Entw'mech., **47**:119.

SCHULZE, W. 1930. Der Einfluss der inkretorischen Drusen und des Nervensystems auf Wachstum und Differenzierung. In Bethe *et al.* Handb. d. Norm. und Path. Physiol., **16**:II/I:699.

SHUMWAY, W. 1917. The effects of a thyroid diet upon *Paramecium*. Jour. Exper. Zoöl., **22**:529.

SWINGLE, W. W.; HELFF, O. M.; and ZWEMER, R. L. 1924. The effect of thyroxin and its acetyl derivative on amphibians and mammals. Amer. Jour. Physiol., **70**:208.

TERAO, A., and WAKAMORI, N. 1924. Preliminary note on the effects of thyroid feeding on the silkworm. Japan. Med. World, **68**.

TORREY, H. B. 1923. The effect of dissolved thyroxin on the division rate of *Paramecium*. Anat. Rec., **26**:367.

TORREY, H. B., RIDDLE, M. C., and BRODIE, J. L. 1924. Thyroxin as a depressant of the division rate of *Paramecium*. Jour. Gen. Physiol., **7**:449.

UHLENHUTH, E. 1921. The internal secretions in growth and development of amphibians. Amer. Naturalist, **55**:193.

WOODRUFF, L. L., and SWINGLE, W. W. 1922–23. The effects of thyroid products on *Paramecium*. Proc. Soc. Biol. and Med., **20**:386.

WULZEN, R. 1916. Pituitary gland: its effect on growth and fission of planarian worms. Jour. Biol. Chem., **25**:625.

THE EFFECT OF DEAD OPTIC VESICLE UPON EXPLANTS OF PROSPECTIVE ECTODERM

(Five figures)

C. D. VAN CLEAVE
Department of Anatomy, University of Pennsylvania, Philadelphia
Pennsylvania

THE circumstances surrounding the formation of the lens vesicle of the eye from the ectoderm under the influence of the underlying optic vesicle suggest that a chemical factor is concerned in this classical case of dependent development. Lewis (1904) discussed the possible chemical nature of the stimulus arising in the optic vesicle and suggested a pertinent approach to the problem: "In the example of the lens reaction it seems evident that the living ectodermal cells must be acted upon, but it is not so evident that the living optic vesicle cells are necessary, as it may be possible to extract from them substances which will give the lens reaction." All investigation of the properties of the dead optic vesicle, however, was destined to wait upon the discovery and elucidation of the properties of the more spectacular dorsal lip of the blastopore. The work on the organizer led to its attempted analysis from the chemical viewpoint. Following the announcement in 1932 (Bautzmann, Holtfreter, Spemann, and Mangold, 1932) of induction effects obtained from organizers killed in various ways, a field more comprehensively covered later by Holtfreter (1933, 1934*a*), came the simultaneous attempts by Spemann, Fischer, and Wehmeier (1933) and by Waddington, Needham, and Needham (1933) to isolate the active principle involved in induction from dead materials and to determine its chemical nature. The inductive potency of variously prepared extracts was successfully demonstrated (reviewed by Weiss, 1935), but the conclusion that a particular substance is the inductive principle is at present controversial.

The work reported in the present paper was originally undertaken in the attempt to apply to the lens problem the methods used in the study of the chemical nature of the organizer. The problem under consideration was the effect of dead optic vesicle and of extracts of dead vesicle upon living presumptive epidermis and upon body ectoderm. During the period in which I have been engaged in the problem, Lopashov (1935, 1936) reported results obtained from a similar approach. He had adopted as a working hypothesis the idea that specific substances are concentrated in the tissue of an organ at the time of its origin. These specific substances would consequently be excreted in abundance by a dead rudiment and should induce in the ectoderm of a gastrula chiefly rudiments like the dead inductor. His experiments consisted of transplanting dead optic vesicles from *Amblystoma mexicanum* and *Triton taeniatus* into the blastocoel of early gastrula stages of *T. taeniatus*. Explants of dead optic vesicles enveloped in a piece of the prospective ectoderm of the same early gastrula stages were also used. Transplantations of living optic vesicles at the same stage served as control experiments. In both cases from one to seven optic vesicles were transplanted.

The dead optic vesicles in both whole embryos and in explants induced rudiments consisting chiefly of eye tissue. A quantitative effect was evident in that one dead optic vesicle gave no induction, whereas 2–7 dead vesicles always induced eye rudiments. Seven inductions were obtained in explants and seven in whole embryos. The eyes induced in the explants were irregular, having the appearance of hollowed outgrowths of retinal tissue. In almost every case they were arranged symmetrically on both sides of a small brain. In one explant two small lenses appeared attached to the ectoderm. In whole embryos the inductions are said to resemble regular eyes well isolated from the brain rudiment. Each eye had induced a lens in the overlying ectoderm. When 7 dead eye vesicles were transplanted simultaneously, three eyes were induced in the explant. Living optic vesicles induced lenses from gastrula ectoderm, but no brain or eye rudiments.

A successful demonstration of the specific effect of a dead inductor would be important in its consequences if it could be generally extended, although it would conflict with previous experience in a far from thoroughly explored field. A very brief summary may serve to orient this aspect of the problem in the general field of amphibian organization. In the first place, the cumulative evidence of today indicates that the quality of organized inductions in host embryos is due to specific influences imposed by living grafts on their surroundings. The "head" and "trunk organizers" of Spemann (1931) and Hall (1932), the specific effect of different levels of the archenteron roof when transplanted into indifferent ectoderm (Mangold, 1933*a*), and the specificity of the inductions brought about by regional fragments of the medullary plate (Mangold, 1933*b*) are cases in point. In the characteristic cases of this type of induction there is no question of stimulation or activation of latent responses in the host tissue.

When dead organizers or extracts of dead organizers or other tissues were introduced into host embryos, the quality of the organized inductions obtained was not determined by the inductor. Weiss (1935) commented: "This consistent lack of specificity in the effects of dead organizers is a general observation, apparently one of the very few features which the bewildering number of inductors, examined so far, have consistently in common." He believed that the organizing principle in these experiments with dead inductors in host embryos was not provided by the "inductor" but by the reacting ectoderm, or, in other words, "by activated fields of the host." The strongest argument for this view was the fact that the quality of the inductions corresponded approximately to their location in the host germ. The fact that living organizers produced specific effects while dead organizers or tissue did not is generally acknowledged; it is in the interpretation of this fact, as seen in Weiss's emphasis on the role of the host fields, that differences arise. Holtfreter (1934*a*) attempted to account for the diversity of effects by postulating a corresponding variety of inductive substances.

The relation of the type of induction obtained in isolated fragments of prospective ectoderm under the influence of dead inductors (Holtfreter, 1933, 1934; Lopashov, 1936) to the foregoing two types of induction is at present obscured by conflicting results. Holtfreter found that in a neutral medium such explants merely proliferate and form epidermis. If brought into contact with a dead inductor they differentiate into neutral tissue, tubes, and vesicles of neural character, but without organization into recognizable parts. Under one condition, when coagulated embryonic chick extract was used as inductor, development of chorda and somites was observed, as in Bautzmann's (1929) experiments with interplantation of ectodermal explants into the eye

orbit. Lopashov's results, as described above, indicated a specific inductive effect upon explanted prospective ectoderm.

The present paper reports results which are interpreted as confirming, on the whole, the idea of specific inductive effect on the part of a dead inductor in explanted prospective ectoderm. The influence of the dead optic vesicle on the differentiation of body ectoderm from the neurula stage and on the prospective ectoderm of the early gastrula was studied in the embryo of *Amblystoma punctatum.*

MATERIAL AND METHODS

The eggs of *A. punctatum* were used exclusively in these experiments. All operations were done under the binocular dissecting microscope with the aid of fine steel needles. The dead material required was prepared by removing the optic vesicles cleanly from early tail-bud stages in which the overlying ectoderm was not yet adherent to the vesicle. With a micro-pipette the vesicles were dropped into 95 per cent alcohol and later dried by placing the dish at 60° C. overnight. This procedure produced a firm, discrete mass of dead material representing one optic vesicle which could easily be picked up as a whole by a needle. Vital staining to identify the dead vesicle was found to be unnecessary; the alcohol fixation of the dead vesicle contrasted in the later staining of the explant fixed with formalin saturated with mercuric chloride. The sharp outline of the dead vesicle in stained sections of the explant made it easily recognizable.

An extract of dead optic vesicle was prepared as follows: optic vesicles were removed from some 25–30 embryos of the usual stage, ground up with anhydrous sodium sulphate and placed in a desiccator until dry. This material was extracted with ether for 48 hours at 30° C. and then filtered. Droplets of oily sediment remained in the filtrate after the ether was evaporated off. This was dissolved in 1 cc. of equal parts of acetone and ether; a small quantity of warm egg albumin and one drop of nile-blue sulphate were added and the mixture coagulated over hot water. Gentle heating was continued until no smell of either ether or acetone was detected. Suitably small pieces of this coagulum were placed between pieces of body ectoderm.

The ectoderm used to test the inductive potency of the dead vesicle was taken from U-shaped blastopore and yolk-plug stages (Harrison's Stage 11 and 12, respectively); body ectoderm from neurulae was used to test the extract preparations and the dead vesicle. A technique for the maintenance of sterility was followed. In order to provide as neutral an environment for the vesicle as possible, free of possible correlative factors generated at a distance and spread over the surrounding field where they might complicate the effects induced by the test object, the explantation method described by Holtfreter (1931, 1933) was adopted and used exclusively in these experiments. A piece of presumptive belly epidermis was removed from the embryo and freed of yolk if necessary. If a single dead vesicle was to be placed in the explant, this piece of ectoderm was cut in two and the vesicle put on one piece and covered by the other, like the meat in a sandwich. If the enveloping ectoderm was left in a single piece and turned like an omelette to cover the vesicle, extrusion of the latter usually occurred. For two or more dead vesicles two pieces of ectoderm from two embryos were necessary to cover the dead material. Fusion of the edges took place in about an hour, and the explant was then transferred to a covered, flat Stender dish, half full of sterile salt solution (Holtfreter, 1931, 1933). The explant rested on a piece of lens paper.

After seven days of cultivation the explants were killed and stained *in toto* with

alum carmine-picric acid solution. Since the diameter of the average explant was about 1 mm., early staining facilitated its handling through vials of dioxan and paraffin.

EXPERIMENTAL

EXPLANTS OF ISOLATED PRESUMPTIVE EPIDERMIS

Explants of presumptive epidermis alone, which had not been in contact with either living or dead optic vesicle, constituted one type of control experiment. A series of 25 such explants was made of presumptive belly epidermis from early gastrulae and, after 7 days' cultivation in the usual sterile salt solution, killed for sectioning. The 16 explants sectioned showed only epidermis—no nervous tissue of any kind. The often irregularly arranged masses consisted of isodiametric epidermal cells among which many cysts of various sizes appeared to indicate secretory activity on the part of the epidermal cells under cultivation (Fig. 4). Similar explants of presumptive epidermis from neurulae also yielded typical epidermal balls. Holtfreter (1933) pointed out that when the ectoderm fastened itself to the glass bottom of the dish instead of rounding up into a ball, as it does when kept away from the bottom, a special case appeared. Then its surface was smoother and the cell mass more compact. In either case, however, the end product of its differentiation was pure, irregularly arranged epidermis.

EXPLANTS CONTAINING EXTRACTS OF DEAD VESICLES

Small masses of coagulated egg albumin which had been impregnated with the ether and acetone extract of a quantity of dead optic vesicles were placed in explanted fragments of the body ectoderm of neurulae. The results were uniformly negative; the epidermis showed no signs of a lens reaction. In some explants very extensive cavities appeared and the surface epidermis was markedly thickened over a large area. No localized area of proliferation was observed and no trace of induced neural structures. Dead optic vesicles in explanted body ectoderm of this stage likewise induced neither a lens reaction nor structures of any kind.

EXPLANTS CONTAINING TWO OR MORE DEAD OPTIC VESICLES

The effect of increasing the number of dead optic vesicles in each explant was studied in explants of prospective ectoderm; a series with two dead vesicles to an explant will be considered first. Three explants made with ectoderm from early gastrulae of Stage 11 died within 3 days; but of 12 explants made with ectoderm from yolk-plug stages, 4 appeared to be alive on the seventh day and were sectioned. One was so disintegrated as to be useless; another may have been dead when fixed but its interior was filled with neuroid masses. Since neither of the dead optic vesicles was identified, their extrusion may have played a part in the survival of the explant. The same picture of necrotic conditions was to be seen in the third explant and the same absence of dead optic vesicles, but the induced masses were here organized into a thick-walled neural structure surrounding a central canal. The fourth explant contained one of its original two dead vesicles in a partially extruded condition and also a large neuroid mass of no discernible organization.

Six dead optic vesicles were placed in each of 2 explants of presumptive epidermis from early gastrulae (Stage 11). The enveloping ectoderm had completely disintegrated by 48 hours.

A series of 8 explants, each of which contained 5 dead optic vesicles, was prepared from ectoderm of yolk-plug gastrulae (Stage 12). These explants went to pieces in 5 days; the optic vesicles were often recognizable as compact bodies lying in the cellular debris of dead ectoderm. Other vesicles had been extruded from the explants as early as the second day.

The fate of these 25 different explants appeared to warrant the conclusion that the early gastrula ectoderm of *A. punctatum* could not survive close contact with large amounts of dead material. The lethal effect of 5 or 6 dead optic vesicles on responsive ectoderm was made evident before any inductive effect could be studied. The mortality of explants with only 2 dead vesicles was impracticably high, but the inductive effect observed in the 3 explants where complete or partial extrusion of the dead material had apparently allowed the survival of the piece suggested that 1 dead optic vesicle might be tolerated in the explant.

EXPLANTS CONTAINING ONE DEAD OPTIC VESICLE

The prospective ectoderm of explants containing 1 dead optic vesicle gave no indication of being adversely affected by this amount of dead material. It responded to the stimulus of the vesicle with some form of neural differentiation in slightly over half the total number of surviving explants. The specificity of this response is, however, the point in question at the present time, and it may be indicated in advance of detailed consideration that of 17 explants showing varying degrees of neural differentiation, only 3 can be said with any likelihood to have responded with the differentiation of an eye rudiment.

Table 1 summarizes the effects induced by 1 dead optic vesicle in explants made of prospective ectoderm from 2 early stages, the U-shaped blastopore and the yolk-plug stage (Harrison's Stages 11 and 12, respectively). In 3 instances the dead vesicle was not found within the explant, nor in its vicinity. Presumably it had been extruded soon after explantation; the ectoderm differentiated into typical pure epidermis. In 13 explants the dead vesicle was identified within the explant and yet the epidermal character of the ectoderm was unchanged. The resulting appearance of the explant was that of a typical epidermal ball. In 5 of these 13 explants the dead vesicle was partially extruded, but the other 8 in which it was completely covered by the epidermal envelope were the same in appearance. Moreover, neural differentiation was observed in other explants where partial extrusion had occurred. Actual contact between the ectoderm and the dead material was apparently not a factor in induction; in many explants the dead vesicle was surrounded on all sides by masses of cells in the closest contact, while in others it lay within a considerable cavity.

In all the remaining explants the dead optic vesicle was identified within the explant and some degree of neural differentiation had resulted. In 5 cases the neural character of the differentiation was evident, but the degree of organization was so slight that the resulting areas could only be called neuroid in character. The change in the nuclei of the cells was particularly marked and an elongation of cell and nucleus was distinct. Tightly packed groups and masses of cells showed indistinct radial arrangement in contrast to the haphazard scattering of cells in other regions of the explant. Sharply defined crevices appeared bordered by orderly rows of elongated cells, but recognizable neural formations were lacking.

In 9 explants the evident neural character of the differentiation was largely confined

to formation with sharply defined boundaries. The structures formed were bizarre and incomplete, as, for example, two well-formed neural tubes which fused into a large neural mass with no central cavity. In general, the amount of tissue involved was small compared with the size of the explant as a whole, but in one case a mass of fused neural tubes almost filled the interior of the explant, while at the other extreme of size was a small neural tube which lay in a large cavity at one end of the explant. In all cases the induced structures were neural in character, either short neural tubes, irregular plates and folds, or unrecognizable brain masses. Organization in the sense of an orderly arrangement of parts was lacking. Nothing of mesodermal or entodermal origin was seen; no structure resembling notochord, gut, or somites was observed.

TABLE 1

THE CONDITION OF EXPLANTS WITH 1 DEAD OPTIC VESICLE AFTER 7 DAYS' CULTIVATION, AS SEEN IN SECTION

	Stage 11	Stage 12
Dead optic vesicle wholly extruded; epidermal ball with no indication of neural differentiation	1	2
Dead optic vesicle identified in explant; epidermal ball with no indication of neural differentiation	6	7
Dead optic vesicle identified in explant; differentiation consists of neuroid masses	4	1
Dead optic vesicle identified in explant; differentiation consists of neural tubes and/or parts of brain	8	1
Dead optic vesicle identified in explant; differentiation consists of brain masses and optic rudiments	0	3
Explants lost in transfer or dead by seven days	3	5
Total	22	19

Three explants, which, in the sense of representing definitely neural rudiments, might be included among the foregoing class are dealt with separately because they presented one feature either missing or extremely obscure in the other explants. This feature was a structure connected with a brain mass which resembled an optic vesicle in general configuration and cellular arrangement.

Explant 112 presents the best example. The induced neural masses lie within a large cavity bounded on three sides by a thin layer of epidermis (Fig. 3). The dead optic vesicle lies at the opposite end of the explant, a considerable distance from the induced mass and does not appear in the section shown. Figure 1 represents an attempt to show graphically the aspect of the entire induced mass in 3 dimensions. Each figure represents roughly one-half the whole mass, although a section slightly larger than the usual interval of 32 μ is unrepresented in the middle of the mass and is responsible for the difference in profiles. The stippled area represents the extent of the hollow interior at the level of the last section in each half. The large round mass of closely packed cells at no time possessed a cavity and shows no connection with the larger mass with which it is in contact at some levels. The structure resembling the optic cup appears at one end of

the larger mass from which it is separated by a deep constriction. The two cavities seen in the photomicrograph are continuous at some levels. Two small round objects composed of radially directed wedge-shaped cells resemble early stages of lens vesicles; one of them is apparently continuous with the large neural mass. For comparison with noninduced brain rudiments grown in explant, a secton through a control explant which shows a lens in process of formation is included (Fig. 5). This explant consisted of a section of the anterior end of one neural fold which had not completely fused with the opposite fold, enveloped in late gastrula ectoderm. The brain rudiment was alive when explanted and included the rudiments of optic vesicle of that side. The photomicrograph (Fig. 5) shows the condition of the rudiment at the end of 3 days' cultivation, a period during which a lens vesicle has formed in the enveloping ectoderm. The lens vesicle is the only induced structure produced in the explant. In the absence of lens fibers at this

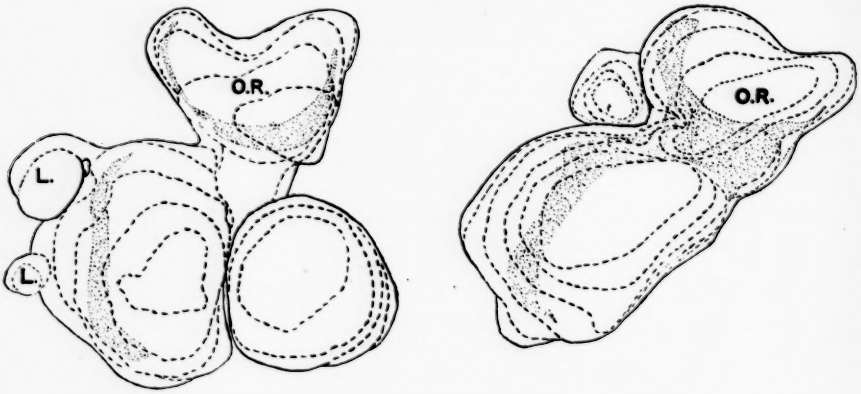


FIG. 1.—Graphic representation of the two halves of Explant 112, a section of which is shown in Figure 3. The dotted lines indicate the outlines of the structures as seen in every fourth section (32 μ). The stippled area represents the extent of the hollow interior at the final level shown; a narrow middle section is unrepresented and accounts for the difference in profiles.

stage of development, the identification of such lentoid structures as seen in Figure 3, with the lens in the control, must be tentative.

Explant 72, a section through which is seen in Figure 2, also presents a mass of induced brain parts and a structure like an optic cup. The dead optic vesicle does not appear in the section. The large neural tubelike structure is more intimately connected with the other hollow rudiment at other levels but the solid mass below has no connection with other structures. No lentoid structures appear in any section. Explant 73 was not in the best condition when fixed, but the sections do show a central brain mass from either side of which protrudes a structure interpreted as an optic vesicle. Associated with one of these induced optic vesicles is a structure which may be a lentoid.

Another series of 16 explants consisting of 1 dead optic vesicle in yolk-plug stage ectoderm but not included in Table 1 may be briefly mentioned. Four explants were killed at the end of 3, 4, 5, and 9 days in the attempt to follow the successive stages in the development of the induced structures. Of the 4 killed after 3 days' cultivation,

2 had extruded the optic vesicle and showed no organization. In the other 2 the cells surrounding the dead vesicle were arranged in concentric whorls and, for a short distance, in orderly rows facing a considerable cavity; otherwise the appearance was much like a typical epidermal ball. Among the explants killed after 4 days was 1 showing definite neural masses, although the dead vesicle had been extruded; this was also true in another killed after 5 days. Another of the same age showed the condition of concentric whorls of closely packed cells seen in a less marked form in 3-day explants. If this condition was preliminary to definitive organization, there was nothing to account for its later appearance here when a 4-day explant had exhibited definite neural masses. The 9-day explants showed only masses of neuroid tissue.

Although the low percentage of inductions defeated the purpose of this series, it is evident that morphological changes which may result in neural structures or which, at the very least, are not epidermal in character, are under way after 3 days' cultivation of the explant. It cannot be concluded from the evidence at hand that the same processes, if continued 24 hours, would produce the definitely neural structures found in the 4-day explant. That the neural induction appeared at all in an explant from which the dead vesicle had been extruded indicates that the continued presence of the latter is unnecessary for induction and suggests that a minimum period of contact might be established.

DISCUSSION

The present status of knowledge concerning the effect of dead inductors in whole embryos and in explanted indifferent ectoderm has been adequately indicated for present purposes in the introductory section. Wide generalizations as to the theoretical implications of qualitatively specific induction seem premature and hazardous in view of the few and conflicting reports on this aspect of the problem of amphibian organization. This discussion will be confined to the experimental results presented above and their relation to similar work.

The positive evidence adduced in this paper for the existence of a specific inductive effect by a dead inductor consists in the induced formation of optic rudiments in isolated fragments of presumptive epidermis which enveloped a dead optic vesicle. The fact that this specific induction occurred in only 3 explants is of secondary importance; it would appear easier to explain why the effect failed to appear than why it did. Some importance can also be attached to a sort of negative evidence, namely, that dead eye rudiment induced eye rudiment and not other neural structures, such as nasal placode and ear vesicle. Further experiments using these rudiments as inductors would appear to constitute valuable and critical controls. In a brief communication Lopashov (1937) stated that he had obtained inductions of nasal placode and ear vesicle from like dead rudiments.

A possible explanation of the gradation of inductive effects seen in Table 1 may be found by postulating a difference in intensity or amount of a hypothetical inductive substance present in the dead eye rudiments. Some explants showed no signs of response to the enveloped dead inductor and differentiated as epidermis; others showed sufficient change in the neural direction to be called neuroid; in still others the induction was definitely neural in character and formed recognizable neural structures; but in only 3 explants were eye rudiments produced in addition to the induced neural structures. The gradation in effect may reflect subminimal and increasing amounts of inductive

substance. Holtfreter's results emphasize that the inductive effect is not of the all-or-none character. Dead inductors and tissues from different sources showed definite gradations in the effects produced. The materials classified as "strong" by him induced a wide diversity of derivatives of all 3 germ layers; the "weak" induced ectodermal derivatives only.

A point of difference between Lopashov's results and my own was the quantity of dead optic-vesicle material necessary for induction. He obtained no induction in either whole embryos or explants with only 1 dead vesicle; at least 2 were necessary for the effect, while with 7 dead vesicles eyes were induced. My experience with *A. punctatum* was that quantities of more than 1 dead vesicle were lethal. This susceptibility may, of course, be a specific difference between the animals used. The importance of the quantity of inductor necessary for induction in *A. mexicanum* and *Triton* gives support to the suggested quantitative explanation of my results.

The part played by the responding ectoderm in these experiments was critically delimited by employing the explanation method. The fragments of prospective ectoderm, isolated in a neutral medium, would appear to provide an ideal proving-ground for inductive effects. They are isolated at the very beginning of gastrulation, have apparently unrestricted potencies (Holtfreter, 1933, 1934*b*), and, in contrast with the same areas *in situ* during development of the whole embryo, are free of the criticism that the spread of various overlapping "fields" of the host has influenced or dictated the nature of the inductions. The salient point of a specific inductive effect is that the characteristic quality of the induced rudiment is imposed upon the responding tissue by the inductor. There can be no question of mere activation or stimulation of a latent response which is evoked by a diversity of inductors. Although I have not yet tried the effect of other dead inductors of a narrowly circumscribed nature, I believe that the results of my experiments indicate that the influence of the dead optic vesicle is imposed on the indifferent ectoderm end so constitutes true induction rather than activation.

SUMMARY

1. The influence of dead optic vesicles and of extracts of dead vesicles upon explants of prospective ectoderm of early gastrulae and of body ectoderm from late neurulae of *A. punctatum* was studied in these experiments.

2. An ether extract of dead optic vesicles induced neither lens nor other structures in explanted body ectoderm of neurulae.

3. Explanted fragments of prospective ectoderm differentiated as epidermis.

4. Explants of prospective ectoderm containing 2 or more dead optic vesicles were killed by this amount of dead material.

5. Explants of prospective ectoderm containing 1 dead optic vesicle showed inductions of definite neural character after 7 days' cultivation. Pattern and organization were lacking in the induced masses; entodermal and mesodermal derivatives were not observed. In 3 such explants were found structures resembling optic rudiments connected with a brain mass.

6. These optic rudiments induced by dead optic vesicle are adduced as evidence of a specific inductive effect of the dead inductor upon explanted presumptive epidermis.

7. The relation of the results of these experiments to other similar work is discussed.

LITERATURE CITED

BAUTZMANN, H. 1929. Ueber bedeutungsfremde Selbstdifferenzierung aus Teilstücken des Amphibienkeimes. Naturwiss., **17**:818.

BAUTZMANN, HOLTFRETER, SPEMANN, and MANGOLD. 1932. Versuche zur Analyze der Induktionsmittel in der Embryonalentwicklung. Naturwiss., **20**:971.

HALL, E. K. 1932. Die Wirkung regionaler Verschiedenheiten im Organisationszentrum. Roux's Arch., **127**:573.

HOLTFRETER, J. 1931. Ueber die Aufzucht isolierter Teile des Amphibienkeimes. II. Züchtung von Keimen und Keimteilen in Salzlösung. Roux's Arch., **124**:404.

———. 1933. Nachweis der Induktionsfähigkeit abgetöteter Keimteile. Isolations- und Transplantationsversuche. *Ibid.* **128**:584.

———. 1934*a*. Der Einfluss thermischer, mechanischer, und chemischer Eingriffe auf die Induzierfähigkeit von Triton-Keimteile. *Ibid.*, **132**:225.

———. 1934*b*. Ueber die Verbreitung induzierender Substanzen und ihre Leistungen in Triton-Keim. *Ibid.*, p. 307.

LEWIS, W. H. 1904. Experimental studies on the development of the eye in Amphibia. I. On the origin of the lens. *Rana palustris*. Amer. Jour. Anat., **3**:505.

LOPASHOV, G. V. 1935. Induction of the eye by a specific substance in the Amphibia. Nature, **136**:835.

———. 1936. Eye inducing substances (English summary). Biol. Zhur. **5(3)**:463.

———. 1937. Ueber die Spezifität induktiver Einflüsse. Compt. rend. (Doklady) acad. sci. USSR, **15**:286.

MANGOLD, O. 1933*a*. Ueber die Induktionsfähigkeit der verschiedenen Bezirke der Neurula von Urodelen. Naturwiss., **21**:761.

———. 1933*b*. Isolationsversuche zur Analyze der Entwicklung bestimmter Kopforgane. *Ibid.*, p. 394.

SPEMANN, H. 1931. Ueber den Anteil von Implantat und Wirtskeim an der Orientierung und Beschaffenheit der induzierten Embryonalanlagen. Roux's Arch., **123**:389.

SPEMANN, FISCHER, and WEHMEIER. 1933. Fortgesetzte Versuche zur Analyze der Indukionsmittel in der Embryonalentwicklung. Naturwiss., **21**:505.

WADDINGTON, NEEDHAM, and NEEDHAM. 1933. Physico-chemical experiments on the amphibian organisation centre. Nature, **132**:239.

WEISS, P. 1935. The so-called organizer and the problem of organization in amphibian development. Phys. Rev. **15**:639.

PLATE I

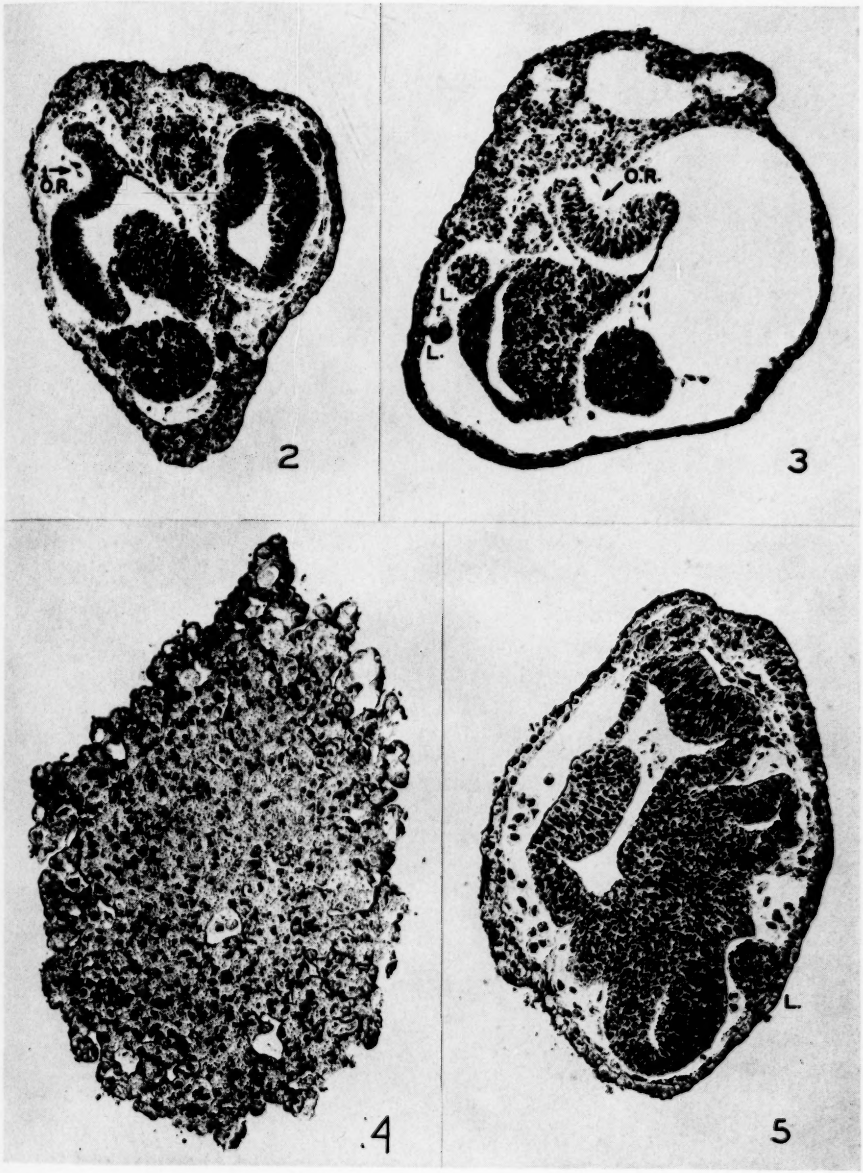
FIG. 2.—Photomicrograph of a section of Explant 72 (× 90) showing neural structures induced by one dead optic vesicle. *O.R.* = optic rudiment.

FIG. 3.—Photomicrograph of a section of Explant 112 (× 90) showing neural structures induced by one dead optic vesicle. *O.R.* = optic rudiment; *L.* = lentoid.

FIG. 4.—Photomicrograph of a section of Explant 87 (× 90) showing epidermal appearance of explanted presumptive epidermis after 7 days' cultivation.

FIG. 5.—Photomicrograph of a section of Explant D 25. Anterior section of a neural fold explanted in the living state after 3 days' cultivation. An optic rudiment has induced a lens vesicle (*L.*) in the ectoderm.

PLATE I



UNIV. OF MICH.

OXYGEN AS A CONTROLLING FACTOR IN THE REGENERATION OF TUBULARIA

(Four figures)

L. G. BARTH
Department of Zoölogy, Columbia University and the Marine Biological Laboratory
Woods Hole, Massachusetts

IT WAS suggested by Morgan (1903) that the inhibition of regeneration when the cut end of *Tubularia* was placed in sand was due to lack of oxygen. Child (1927, 1928) has shown that small pieces of *Corymorpha* in contact with the bottom of a dish develop hydranths from the free surface and stolons from the surface in contact with the glass. He suggests that the differential exposure of the surfaces of the fragment results in a difference in respiratory exchange which may determine the new polarity. There has been no attempt to study quantitatively the relation between oxygen tension and regeneration in *Tubularia* or *Corymorpha*. This paper will discuss, first, some qualitative observations on regeneration of ends of stems of *Tubularia* under conditions of reduced oxygen supply, and, second, a quantitative study of the size of the hydranth, time for regeneration, and rate of regeneration at various oxygen tensions. The experiments were carried out with the very able assistance of Mr. John Moore, who determined the oxygen tensions. The work was supported by a grant from the Rockefeller Foundation.

METHODS

The methods used in measuring rate of regeneration are similar to those used previously (Barth, 1938). After selection of the stems, random samples are taken for each lot and measurements of the diameter of the stem and length of the primordium of the hydranth are made when the constriction appears between the primordia and the rest of the stem. The time in hours required for the appearance of this constriction is termed t_1. The length of the primordia in micra divided by the time in hours is used as a measure of rate of regeneration in these experiments. The rate of regeneration is then measured in micra-hours or regenerative units which we shall designate R.U. For the work on oxygen tension the stems were placed in sea water at the tension to be examined, in flasks of 1,000-cc. capacity and the surface of the sea water covered with a thick layer of nujol. Siphons for taking of samples were inserted through the oil layer. With 20 stems in this large volume of water the O_2 tension did not vary over the period of the experiment. The oxygen tension was determined by Winkler's methods; usually the average of three samples taken at intervals was used.

PRELIMINARY STUDIES

In some of the studies on regeneration of cut stems of *Tubularia* it was found that when the tissue was pinched off for a distance of a few millimeters from the cut end of the perisarc no regeneration occurred. It appeared then that the tissue must be at the opening of the perisarc in order to differentiate into a hydranth. This inhibition of regeneration when the tissue is not at the opening of the perisarc might be due to lack of an

adequate oxygen supply, as the diffusion rate in small capillaries such as the tube formed by the perisarc is very slow. The perisarc averages about 0.5 mm. in diameter. On the other hand, waste products might accumulate for the same reason.

Further experiments, in which the rate of regeneration was measured with the distal end of stems placed in glass capillaries about 1 mm. in diameter, showed that complete inhibition of distal regeneration occurred, with the further result that the proximal ends regenerated more rapidly (Figs. 1, *A*, *B*, *C*). The results are clear. There is no regeneration at the end which is capped with a glass capillary, while when the capillary is slipped over the stem, leaving the ends free, almost the normal rate of regeneration is found—32.1 R.U. as compared with 38 R.U. at the distal end and 4 R.U. compared with 2.7 R.U. at the proximal end. The stems used for these experiments were short, 10 mm., and thus there was almost complete dominance of the distal end over the proximal end

	A		B		C	
	D	P	D	P	D	P
L	0	912	1088	156	1244	100
r	0	240	242	200	244	340
t_1	–	42.8	33.9	39.0	32.8	37.0
R	0	21.3	32.1	4.0	38.0	2.7

FIG. 1.—Inhibition of regeneration by covering the cut end of *Tubularia* with a glass tube. Stems 10 mm. long; 20 stems in each group, *A*, *B*, and *C*. L = length of primordium in micra; r = radius of cross-section in micra; t_1 = time in hours from cutting to the formation of the primordium; R = rate of regeneration as measured by L/t_1. Group *A*. the distal end is inserted in a glass tube; Group *B*, the middle part of the stem is covered by the ends are free; Group *C* serves as untreated control; *D* = distal end of the stem; *P* = proximal end of the stem.

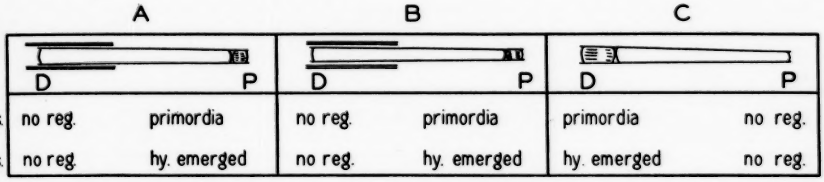
in *B* and *C*. However in *A*, where regeneration of the distal end was prevented by the glass cap, the rate of regeneration of the proximal end rose to 21.3 R.U.

Examination of the distal ends within the tubes showed the coenosarc disintegrating in some cases, leaving this region of the perisarc filled with a mass of unorganized cells and cell debris. In other cases the distal end simply closed after cutting and showed no further signs of either disintegration or differentiation.

The next experiment shows that even when the glass tube is completely open at the end regeneration is inhibited. In this case the cut end was about 0.5 mm. from the opening of the glass tube (Fig. 2, *A*), but no differentiation took place. As is expected, inhibition of the distal end causes the proximal end to regenerate. These results were surprising, and the experiment was repeated with another group (Fig. 2, *B*) in which the distal ends of stems were placed in glass tubes with the cut ends flush with the ends of the tubes. After 24 hours there was no regeneration at the distal end in the tubes while the proximal free ends were regenerating. The controls (Fig. 2, *C*) showed the distal end to be completely dominant in these stems. The results show clearly that covering the end of the stem with an open glass tube inhibits regeneration. Further experiments, using a porcelain plate with small perforations, gave the same effect when the cut ends of stems were placed in the holes.

THE EFFECT OF VARYING OXYGEN TENSION UPON THE REGENERATION OF CUT STEMS

The preliminary studies indicate that reducing the oxygen supply of the cut end by means of glass tubes inhibits regeneration. In the following experiments sea water was made up at various oxygen tensions and twenty cut stems treated. The size of the developing hydranth as measured by the length of the primordium, L, the time for the formation of the primordium, t_1, and the time for emergence of the hydranth, t_2, were observed. The stems used were 5–7 mm. long so that dominance of the distal end was almost complete and few or no proximal hydranths developed at normal oxygen tensions. Since the stems were in sealed containers, they had to be removed for measurement. They were taken out when, by gross inspection, about 50 per cent showed primordia. After removal the stems were kept at the normal O_2 tension of sea water in open dishes. This procedure meant that the stems at higher O_2 tensions were treated for a shorter time than those at lower O_2 tensions because of the difference in rate of regeneration.



	A		B		C	
24 hrs.	no reg.	primordia	no reg.	primordia	primordia	no reg.
48 hrs.	no reg.	hy. emerged	no reg.	hy. emerged	hy. emerged	no reg.

FIG. 2.—Inhibition of regeneration of cut ends of *Tubularia* in glass tubes. A = distal cut end about 0.5 mm. from opening of tube; B = distal end flush with opening; C = control. Stems 10 mm. in length; 20 stems in each group.

Many preliminary experiments were carried out, but the last three experiments of the series will be used chiefly for this paper. The data from these are included in Table 1. Here the oxygen tension is recorded, the average lengths in micra, the average time in hours, and finally the average rate of regeneration of 20 stems is calculated as $R = L/t_1$ = R.U. When a stem completely fails to regenerate, it is not included in the calculations. When a stem fails to regenerate during the experimental period but regenerates later, it is considered to be inhibited and it is included in the calculations. The length of the primordium of these latter will be 0, since it does not appear during the experiments, and the rate of regeneration, 0. This treatment of the data is necessary, as in low O_2 tensions the weaker stems fail to regenerate, while at higher O_2 tensions they are able to do so. Naturally in trying to measure the effect of O_2 on regeneration these weaker stems must also be included.

As seen from Table 1, with increase in O_2 tensions there are an increase in size of the primordium of the hydranth, a decrease in the time for its formation, and an increase in the rate of regeneration which is a function of these two variables. The magnitude of the effect is great, so that the rate of regeneration of stems from the same source ranges from 22 R.U. at 1.3 cc. O_2/l to 67.1 R.U. at 8.6 cc. O_2/l.

The data from Experiment 9 in which the oxygen tension of laboratory sea water was 4.7 cc./l are plotted in Figure 3. All the Experiments 1–8 agree with Figure 3 in showing that the greatest effect of O_2 tension is between 3 cc. and 5 cc. of O_2 per liter. This fact is of great importance since this range includes the normal oxygen tension of sea

water in the laboratory. Any experiments on the rate of regeneration of *Tubularia* must take into consideration this critical range of O_2 tension.

Below 3 cc. of oxygen per liter the rate of regeneration falls off less rapidly, until a tension of about 1 cc. is approached, when the rates of regeneration decline sharply. At about 0.35 cc./l regeneration is inhibited completely, while above 5 cc. the rate continues to increase, but more slowly. The relation between O_2 tension and rate of regeneration from 4 cc. to 21.6 cc./l is approximately logarithmic, as shown in Figure 4. Here the rates of regeneration of Experiments 5, 7, 8, and 9 are plotted as functions of the logarithm of the oxygen tension and show a linear relationship. The older stems are more susceptible to changes in O_2 tension since the slope of these lines is greater than the slope of plots from younger stems. Compare Experiments 5 and 7 with 8 and 9. In Experiments 8 and 9 the stems were so similar that their rates of regeneration fell on the same line.

TABLE 1

THE EFFECT OF VARYING OXYGEN TENSIONS ON THE RATE OF REGENERATION OF *Tubularia*

C = control in open dish

Experiment	O_2 Cc./Liter	L Micra	t_1 Hours	$R = L/t_1$
Experiment 7: 180 stems 5–7 mm. length, young....	2.4	1,072	36.1	29.7
	3.2	1,284	28.1	45.7
	4.1	1,370	26.8	51.3
	4.8	1,365	26.3	52.0
	8.2	1,640	24.5	67.0
	11.3	1,809	24.6	71.8
	14.3	1,840	24.1	75.5
	16.5	1,846	23.7	77.0
	C	1,370	26.2	52.2
Experiment 8: 160 stems 5–7 mm. length, medium...	2.9	990	36.0	27.4
	3.1	1,050	32.5	32.2
	3.3	1,072	34.2	31.0
	3.9	1,192	32.4	36.7
	4.5	1,190	32.1	37.0
	4.9	1,350	27.8	48.5
	6.5	1,412	26.4	53.5
	7.4	1,635	24.9	65.8
Experiment 9: 160 stems 5–7 mm. length, old.....	1.3	1,170	53.3	22.0
	1.8	1,250	48.1	26.0
	2.3	1,260	46.2	27.4
	3.0	1,270	42.7	29.7
	4.1	1,250	35.6	35.2
	4.7	1,420	29.7	47.7
	6.4	1,520	26.1	58.3
	8.6	1,710	25.4	67.1

The lower limit of O_2 tension for regeneration has not been accurately determined, but at 0.35 cc./l the stems may remain indefinitely without regenerating at either end, while at 1.33 cc./l most of the stems regenerate at the distal end, giving a rate of 22 R.U. Thus there must be a rapid drop from 22 to 0.0 R.U. between 1.3 and 0.35 cc. of oxygen per liter.

The highest O_2 tension tried was 21.6 cc./l, in which the stems gave a rate of 57.8 R.U. as compared with a rate of 50 R.U. at 14.8 cc./l. The range from 10 cc./l to 20 cc./l is characterized in all experiments by a flattening of the curve (Fig. 3).

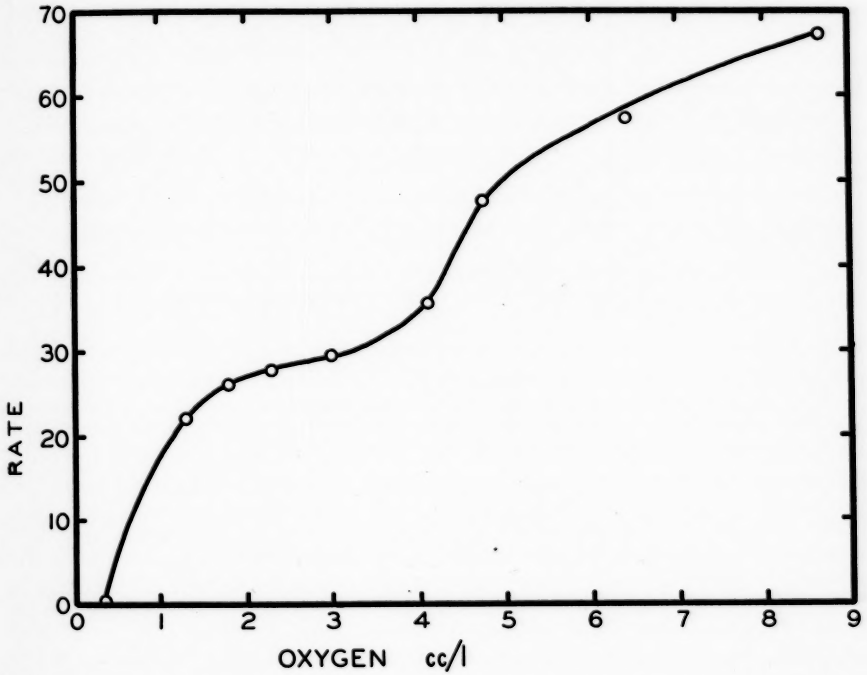


FIG. 3.—The effect of varying the oxygen tension on the rate of regeneration of *Tubularia*. Rate = L/t_1, where L = length of primordium in micra and t_1 = time in hours. Oxygen tension is recorded in cubic centimeters per liter.

The beneficial effects of high O_2 is demonstrated by the stems of Experiment 9. These stems were removed from the flasks containing O_2 at various tensions and placed in a dish of sea water for observation. The distal primordia were measured and the distal hydranths emerged, and then, as is characteristic on standing in unchanged sea water, the hydranths, after about 96 hours, dropped off and stolons grew out of the proximal ends of the stems. After 264 hours there were no hydranths present in the 160 stems, but the stems were not dead, as circulation continued. The stems were then placed in high O_2 of 14 cc./l and in 48 hours almost all the stems formed new hydranths.

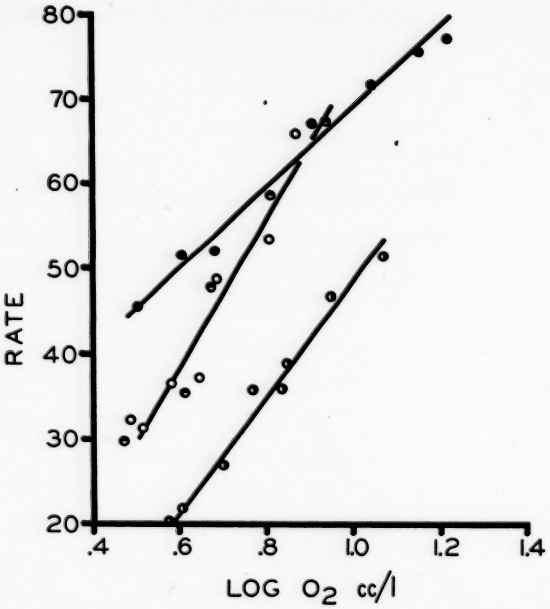


FIG. 4.—Rate of regeneration of *Tubularia* at varying oxygen tensions. Rate $= L/t_1$ in micra/hours Data from four experiments. Curve showing greatest slope is from Experiments 8 and 9 on relatively old stems. The other two curves are from Experiments 5 and 7 on relatively young stems.

TABLE 2

EFFECT OF VARYING OXYGEN TENSIONS ON THE RATE OF REGENERATION OF *Tubularia*

Experiment 2: length 15 mm.; 13 stems in each sample; treated for 40 hr., then removed to open dish for observation; L = length; t_1 = time in hours; $R = L/t_1$ = micra/hours.

	Distal			Proximal		
O_2 Cc./Liter	L Micra	t_1 Hours	$R = L/t_1$ R.U.	L Micra	t_1 Hours	$R = L/t_1$ R.U.
1.3	1,080	114.0	9.1	0	.....	0.0
4.76	1,200	48.3	25.0	564	48.7	11.6
8.08	1,210	48.0	25.4	440	56.0	7.9
13.75	1,730	40.0	43.4	720	50.5	14.3
19.1	1,820	40.0	46.5	980	49.4	20.0

THE EFFECT OF OXYGEN ON DOMINANCE OF THE DISTAL OVER THE PROXIMAL END

In most of the experiments the stems were chosen of such length, 5–7 mm., that almost complete dominance resulted. In one experiment 15-mm. stems were used and proximal hydranths developed in the higher O_2 tensions but not in the lower ones. Table 2 gives these results. At 1.3 cc./l the proximal ends do not regenerate, while at 19.1 cc. the rate is 20 R.U. Observation on Experiment 9 also showed that at O_2 tensions of 8.65 cc./l, 60 per cent of the proximal ends formed hydranths; 15 per cent, stolons; and 25 per cent showed no action. Lowering the O_2 tension to 4.1 cc./l gave 20 per cent hydranths, 50 per cent stolons, and 30 per cent no action. These stems, although 5–7 mm. long, were older than in other experiments and dominance was accordingly less evident.

DISCUSSION

The close dependence of rate of regeneration upon oxygen tension is extremely interesting in relation to studies on the control of polarity in *Tubularia* and other allied coelenterates. In the first place, the earlier results of Morgan (1903) on placing the end of *Tubularia* in the sand and inhibiting regeneration are clearly due to a lack of oxygen. Even in open tubes, as in my experiments, the oxygen is used up so fast that diffusion is unable to supply enough for regeneration. Second, Child's observation (1928) that the free surface of *Corymorpha* fragments always becomes the hydranth can be interpreted on the basis of higher oxygen supply. Third, the lack of regeneration when the ends of *Tubularia* are ligatured is almost certainly due to a lack of O_2, for it is clear that the perisarc is not freely permeable to O_2. This explanation is confirmed by the observation that when the perisarc cracks near a ligature the exposed tissue will start to form a hydranth, i.e., admission of O_2 from sea water allows for regeneration. Further experiments on the stimulation of regeneration by rupturing the perisarc will be reported in a separate paper. It is suggested that the stimulus for regeneration is the exposure of tissue to high oxygen. Aside from the light that these experiments throw upon the mechanism of determination of polarity, the physiological mechanism involved is of extreme theoretical importance. How does increased oxygen tension act? Does it increase O_2 consumption and so perhaps increase metabolism generally so that the processes of regeneration are hastened? Or does the increased O_2 tension simply increase the rate of diffusion of O_2 into the stem so that it penetrates more deeply and thus stimulates more of the stem to form a hydranth? This latter suggestion would account for the larger hydranths at high O_2 tensions, but not the decrease in time for regeneration. The same two possibilities exist for the explanation of the inhibition due to low O_2 tensions.

No data presented in this paper can be used to distinguish between these two possibilities. The relation between O_2 tension and O_2 consumption will have to be determined. Henze (1910) says that the O_2 consumption of two coelenterates, *Actinia* and *Anemonia*, is dependent on O_2 tension, while in two others, *Pelgia* and *Carmarina*, the O_2 consumption is independent of O_2 tension.

We have good evidence that rate of diffusion is an important factor. The experiments cited in this paper show that at the same O_2 tension the ends of *Tubularia* in open tubes will not regenerate while those free in the water will. The only explanation lies in the reduced O_2, owing to utilization by the end and failure of diffusion to supply the minimum tension required for regeneration.

SUMMARY

1. Experiments in which glass tubes were used to cover the cut end of *Tubularia* show that diffusion of O_2 at the tension of sea water, through less than a millimeter of 1-mm. glass tubing is not sufficient to supply the tissues for purposes of regeneration.

2. Varying the oxygen tension of sea water affects the size of the hydranth primordia, the time for formation of the primordia, and the rate of regeneration as measured by those two variables.

3. Increasing the oxygen tension above 4.5 cc./l increases the rate of regeneration rapidly, while decrease below 4.5 cc./l causes a sharp decline in rate. *Tubularia* therefore is existing at a very critical region of the oxygen-tension regeneration curve.

4. The lower limit for regeneration is between 0.35 and 1 cc. of oxygen per liter, at which regeneration is inhibited indefinitely but the stems are not killed. The effect is reversible, regeneration taking place when the oxygen tension is increased.

5. The rate of regeneration above 4 cc. of oxygen per liter is a linear function of the logarithm of the oxygen tension.

LITERATURE CITED

BARTH, L. G. 1938. Quantitative studies of the factors governing the rate of regeneration in *Tubularia*. Biol. Bull. **74**:155.

CHILD, C. M. 1927. Modification of polarity and symmetry in *Corymorpha palma* by means of inhibiting conditions and differential exposure. Jour. Exper. Zoöl., **47**:343.

———. 1928. Axial development in aggregates of dissociated cells from *Corymorpha palma*. Physiol. Zoöl., **1**:419.

HENZE, M. 1910. Über den Einfluss des Sauerstoffdruchs auf den Gaswechsel einiger Meerestiere. Biochem. Zeitschr., **26**:255.

MORGAN, T. H. 1903. Some factors in the regeneration of *Tubularia*. Arch. f. Entw'mech., **16**:125.

DIFFERENTIAL GRADIENT FUNCTIONS IN THE FEATHER GERM[1]

(Eight figures)

RICHARD M. FRAPS
Whitman Laboratory of Experimental Zoölogy, University of Chicago[2]

LILLIE and Juhn (1932) have shown that the localization of pigmentation patterns induced by hormones in saddle and breast feathers of the Brown Leghorn male or capon requires the postulation of a purely quantitative reaction gradient in the collar of the developing germ. The concentration of hormone required to effect a given pigmentation reaction at different regions of the collar was taken as the measure of the underlying physiological gradient that determined observed threshold values. By this measure the high end of the gradient in saddle and breast feathers is at or near the ventral limit of the collar (apexes of barbs), the low end is at or near the dorsal limit of the dollar (bases of barbs). Lillie and Juhn also attempted to show that the underlying, determinative gradient in the collar was primarily a gradient in rates of cell division.

In extending the earlier experimental procedures to other tracts, patterns have been obtained which indicate that the order of pigmentation in the germ is partially or completely the reverse of the order described by Lillie and Juhn as typical in the saddle and breast germs. One might, of course, take these patterns as evidence for a reversal in direction of the underlying gradient differentials postulated for saddle and breast germs. The possible existence of localized reversals in direction of the primary collar gradient has, in fact, been recognized by Lillie (1932) and cannot be excluded in certain pattern formations (penciling, for example). But, if we think of the reaction gradient (for pigmentation) described by Lillie and Juhn as the direct concomitant of primary differentials in developmental processes (e.g., rates of cell division), we must also suppose fundamental differences in the organization of feather germs exhibiting the partially or completely reverse orders of pigmentation described below. In certain germs the high end of the reaction gradient, as measured by hormone concentration required to produce a given reaction, is at the ventral limit of the collar; in others, at the dorsal limit; and in still others the gradient has high (not necessarily equal) values at ventral and dorsal limits with minimal values at intermediate collar levels. The organization of the feather germ and the primary differentials associated with its development can scarcely differ in correspondence with these relations.

Experimentally induced pigmentation patterns in the Brown Leghorn always appear as alternatives to the immediately displaced pigmentation reaction. If we assume that the conditions tending to effect each of these alternatives represent *differential functions* of a primary developmental gradient, such as rate of cell division, the reversal of thresh-

[1] Presented before the National Academy of Sciences, by invitation of Dr. C. M. Child, under title of "Reaction Gradients and Pigmentation Thresholds in the Feather Germ" (abstract in *Science*, **85** [1937]:59).

[2] This investigation was supported in part by funds from a grant by the Rockefeller Foundation to the University of Chicago in aid of biological research.

old differentials in different germs represents only a differing relationship between two critical but derivative processes of graded intensity within the collar limits. The primary developmental gradients in different germs (gradients in rates of cell division, differentiation, etc.) are assumed, on this view, to remain unchanged *in direction* but to differ in absolute values in feather germs differing in growth-rates, reaction orders, etc.

The reaction gradient originally described by Lillie and Juhn in the collar of the feather germ is a most beautiful confirmation of the role that C. M. Child has long attributed to purely quantitative, physiological differentials in the developmental processes. The feather germ is remarkable not only because qualitative differentiations evi-

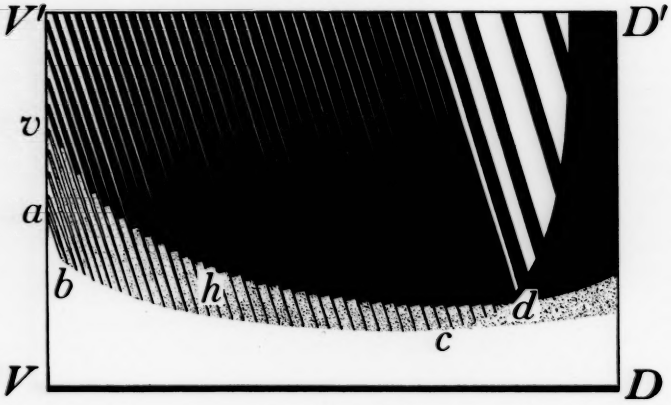


FIG. 1.—Diagrammatic representation of one collar limb from a regenerating feather germ. The germ has been split along the ventral superficial axis, VV', and the center of the shaft, *DD'*; the differentiated apical levels of barbs and shafts above *V'D'* have been removed, and also the undifferentiated base of the germ below *VD*. Barb primordia originate along *ab;* their bases differentiate along the locus *bc* and are completed at *d* with union of barb-shaft structures. Spacing of barbs with growth of shaft is indicated above *d*. Locus of simultaneous pigmentation of barb complement is represented by the locus *vd*. Differentiation and pigmentation of shaft are indicated schematically for completion of the diagram.

dent as different pigmentation products are shown to be localized with respect to quantitative differentials but equally because of the fact that these qualitatively different products are represented in all cells by definite, relatively simple genetic compositions. Here, certainly, is a developmental system for which we may hope eventually to formulate a coherent and rigorous statement of relationship between the discrete date of genetics and the quantitative "organizing" differentials of Child's gradient theory. It is a pleasure to contribute to this number honoring Professor Child a paper which, in common with so much in developmental investigation and theory, must take for granted the pervasive fertility of his conceptions.

1. DEVELOPMENTAL RELATIONS IN THE FEATHER GERM

The several orders of induced pigmentation patterns found in regenerated feathers and described below occur in correspondence with definite relations in the developing

germ. A brief presentation of essential germinal relations is accordingly called for in this place; detailed accounts have been given by Lillie and Juhn (1932), Greite (1934), Hosker (1936), and Fraps and Juhn (1936).

Figure 1 represents a section cut from the cylindrical base of a germ after development is well under way. The cupped, undifferentiated base of the germ has been removed by cutting along *VD;* the apical, completely differentiated portions of barbs and shaft are cut away along *V'D';* and from the annular band thus formed, one-half of the germ producing a single vane-half of the feather is obtained by splitting along the ventral superficial axis (*VV'*) and through the center of the shaft (*DD'*). The developing region of a complete and wholly symmetrical germ would be represented diagrammatically by producing a mirror image of Figure 1 to the left of *VV'* or to the right of *DD'*.

In the barb complement simultaneously represented in the feather germ (from *b* to *d*, Fig. 1), the youngest primordium is at the ventral face of the collar (at *ab*), the oldest is adjacent to the shaft (at *d*). The apex of the individual barb forms first (*ab*), and successive apicobasal levels arise as the primordium formed at *ab* occupies successive positions along the locus, *bc*. At *d* the base of the completed barb has united with the shaft primordium (union occurring between *c* and *d*), and it is carried from the collar by axially directed growth of the shaft. Growth of the shaft, particularly during the second phase of growth (ballooning of Lillie and Juhn) gives to the barbs their definitive spacing on the shaft. This is indicated—altogether schematically—in Figure 1 by the increasing separation of barbs above *d*.

The region of growth by cell division in the germ includes the undifferentiated base (white of Fig. 1) and a limited extent within barb primordia—up to the distal level of cell division, according to Lillie and Juhn. Axially directed growth causes a continual apicalward prolongation of the undifferentiated zone; the simultaneous projection of barb bases into this zone maintains the general relation shown in Figure 1. It follows in consequence of this axially directed growth that the line of cleavage between the undifferentiated basal tissue and the last barb primordia (*ab*) formed from it will be removed from the base of the germ until a succeeding primordium can form in the same relative position occupied by *ab*, Figure 1.

Since the lines of cleavage between barbs form an angle with the vertical axis, *VV'*, barb bases must move, with continued axial growth of the germ and therefore in length of each barb, from *b* to *c*, where processes of union with the shaft begin (Hosker, 1934). In many germs, however, the angle of cleavage tends to approach the vertical as we follow the locus *bc*. This is the same as saying that the angle of cleavage (*Vab* of Fig. 1) decreases with the level of barb bases above a given base line (*VD* of Fig. 1). The general relationship suggests a primary gradient differential from ventral to dorsal limits of the collar, a differential involving possibly growth vectors (Fraps and Juhn, 1936), as well as rates of growth.

The locus of simultaneous pigmentation in all reactive barb primordia is indicated in Figure 1 by the curve, *vd*. Barb apexes are not pigmented immediately upon their formation, but only after they have reached the relatively high level, *v*, above the base of the germ. There are consequently a number of barb primordia at the ventral limit of the germ that are entirely unpigmented.

It follows from the serial order of origin of barb primordia and their ventrodorsal motion around the collar that the simultaneous pigmentation of all barbs cutting the locus *vd* corresponds to pigmentation of the individual barb at successive apicobasal

levels (Lillie and Juhn, 1932). The locus *vd* in the germ appears in the regenerated feather as a line approximately at right angles to the shaft, as *vd* of Figure 2*A–E:* in these representations of single vane-halves, the margin (barb apexes) corresponds to the ventral position in the germ, and barb bases correspond to dorsal position. With these simple relations in mind, any pigmentation pattern in the regenerated feather can be referred directly to the developing germ.

2. EXPERIMENTALLY INDUCED ORDERS OF PIGMENTATION

By "order of pigmentation" we refer to the direction—dorsoventral or ventrodorsal—in which an imposed pigmentation pattern displaces an alternative pigmentation forma-

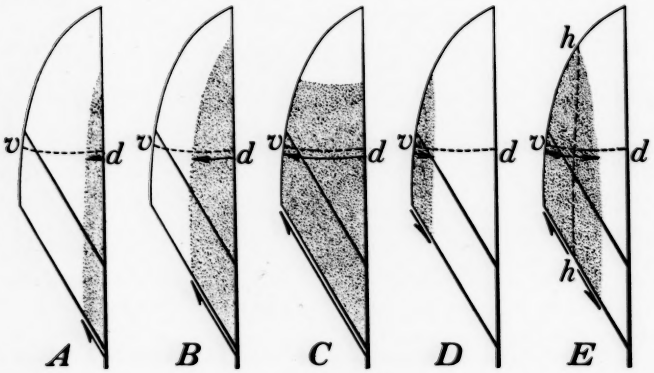


FIG. 2.—Orders of pigmentation in regenerated feathers. Each figure represents a section from a single vane-half with barbs ranged parallel to those shown. The locus of simultaneous pigmentation in the germ (*vd*, Fig. 1) is indicated in each section by the transverse broken lines, *vd*. The arrows beneath *vd* give the direction of imposed pigmentation (stippled regions) with increasing hormone concentrations. The arrows at the base of the sections give order of pigmentation referred to the individual barb corresponding to levels cut by *vd*. *A*, *B*, and *C:* dorsoventral order of pigmentation, typical in collars of breast and saddle feathers in reaction to female hormone and thyroxin, respectively. *D:* ventrodorsal order of pigmentation with increasing hormone concentration (medial vane-half of secondaries in reaction to female hormone). *E:* complex order of pigmentation, proceeding from a boundary, *hh*, ventrally and dorsally (lateral vane-halves of secondaries).

tion with increasing hormone concentration. Three orders of pigmentation have been experimentally produced in feathers of the Brown Leghorn male or capon.[3]

a) *Dorsoventral pigmentation.*—The order of pigmentation of breast feathers to female hormone and of saddle feathers to thyroxin has been described in detail by Lillie and Juhn (1932). The breast feathers may be taken here as illustrative of this order of re-

[3] Of the three pigmentation orders taken up here, only one, the dorsoventral order, has been described in the literature. The ventrodorsal and complex orders of pigmentation were obtained by Dr. Mary Juhn in connection with experiments designed to establish the relation between rate of feather growth and hormone concentration in rapidly growing feathers. These results will be published elsewhere. The author wishes to thank Dr. Juhn for placing at his disposal, prior to publication, the extensive material constituting the experimental basis of this paper.

action. In the male or capon these feathers are solidly black; the subcutaneous injection of female hormone causes the appearance of the brilliantly contrasting "female salmon."

The minimal concentration of female hormone required for the appearance of female salmon in the barbs (leaving out of account the shaft) produces a pattern limited to barb bases. If this relatively low concentration is maintained constant, a longitudinal pattern similar to that indicated in Figure 2*A* is produced (female salmon represented by stipple). This corresponds to reaction at and near the dorsal limit of the germ (to the left of *d*, Fig. 1). A somewhat higher concentration of female hormone maintained at a constant level would produce a larger central area of salmon (Fig. 2*B*), corresponding to reaction in the germ from *d* to *h*. Further increases in the concentration of hormone extend the reaction for salmon pigmentation from *h* toward *v* (Fig. 1) until finally black is displaced

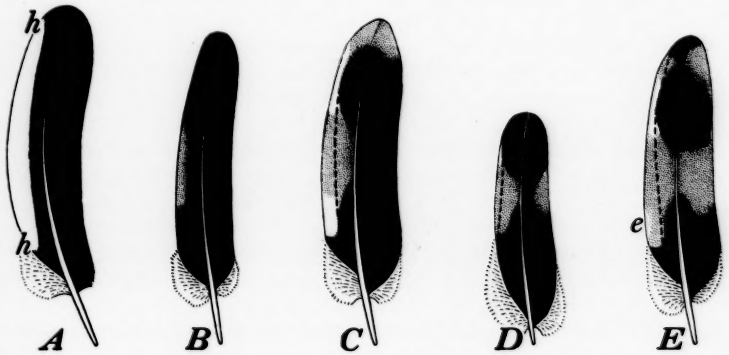


FIG. 3.—Normal and modified feathers from the sequence of secondaries, male or capon Brown Leghorn. *A:* normal feather, black in the figure representing black in the feather, white area corresponding to brown in the feather; *hh*, boundary corresponding in mid-vane regions to *h* in Figure 1. *A*, *B*, *C*, and *D:* increasing degrees of reaction to female hormone (stippled areas of figures) in different feathers of the secondary series. The longitudinal broken lines in Figures *C*, *D*, and *E* correspond approximately to the normal boundary between brown and black. Details in text.

entirely from the locus of pigmentation. The resulting feather is completely salmon below the level at which the requisite hormone concentration has been attained (Fig. 2*C*).

In the breast feather, then, the order of reaction to increasing concentration of female hormone is strictly dorsoventral. The same order of reaction is typically observed in formation of black under the action of thyroxin in feather germs of the back, saddle, and neck hackle regions of the Brown Leghorn capon (details in Lillie and Juhn, 1932).

b) *Ventrodorsal pigmentation.*—The secondaries of the Brown Leghorn male or capon are typically black with a marginal brown stripe in the lateral vane-half of each feather (Fig. 3*A*, black as represented, brown indicated by white in the lateral [left] vane-half). Female hormone displaces both brown and black, inducing formation of the stippling pattern characteristic of the normal female. Pigmentation of the individual stippled barb occurs as an alternate banding of black and buff. The proportion of black is greater toward the base of the barb, the proportion of buff toward the apex.

Taking into account only the main vane region of the secondaries (excluding, that is, apical formations), increasing concentrations of female hormone effect the formation of

stippling in ventrodorsal order *in all normally black regions*. In the medial vane-half (completely black), pigmentation is extended from the ventral limit of the germ to the dorsal limit with increasing hormone concentration (Figs. 2*D* and 3*D*). In the lateral vane-half the extension of stipple with rising hormone concentration proceeds from the boundary between brown and black (*h* of Fig. 1; *hh* of Figs. 2*E* and 3*A*) toward the dorsal limit of the germ, corresponding, of course, with extension toward the shaft in the regenerated feathers (Fig. 3*B*, *C*, *D*, and *E:* observe also that the order of displacement of stipple by black with falling hormone concentration in Fig. 3*C*, *D*, and *E* is from shaft to brown-black boundary).

The most direct evidence that we are dealing with a true ventrodorsal order of reaction in all normally black regions of these feathers lies in the fact that we never find (in the main vane regions) stippled areas adjacent to the shaft with black areas surrounding them. In a few instances the extreme apical levels of barbs in the medial (solidly black) vane-halves remain black even with considerable extension of stipple toward the shaft. But excepting these very limited barb regions, the ventrodorsal relationship appears to be consistently maintained in the displacement of black.

c) *Complex pigmentation orders.*—In terms of relations within the collar of the developing feather germ, the dorsoventral order of pigmentation (breast feathers) and the ventrodorsal (medial, solidly black vane-half of secondaries) both represent continuous reaction gradients. In the lateral brown-black) vane-half of the secondaries, the reaction for stippling is initiated with minimal hormone concentrations at the boundary between brown and black (*h* of Fig. 1; *hh* of Fig. 3*A*); and with increasing concentrations, stippling is extended from this point both ventrally and dorsally (Fig. 2*E*). This is well brought out in the "mirror-imaging" of the induced stippling on opposite sides of the broken line drawn into Figure 3*C*. The only qualification to be made here is that in a considerable number of feathers the entire brown region appears to have become stippled practically simultaneously, with very little encroachment into the region of black pigmentation. In others, however, marginal areas remain unstippled (brown), as at *e*, Figure 3*E*—clear evidence that the ventral region of the germ has maximal threshold values so far as the displacement of brown is concerned.

The general relation between the concentration of hormone required to effect modification in the several collar limbs, and the direction of modification in each collar limb, can be given in a single graph, Figure 4. Abscissas here represent equivalent collar positions, *dv;* ordinates indicate concentrations. Curve *I* represents the relation between hormone concentration and direction of imposed pigmentation (*dv*) in the dorsoventral order. Curve *II* is the same relation for ventrodorsal order of pigmentation, just the reverse of curve *I*. Curve *III* combines portions of these two curves, with *h* the point of modified pigmentation at minimal hormone concentration.

3. DIFFERENTIAL FUNCTIONS OF PRIMARY GRADIENTS

Lillie and Juhn (1932) interpreted their experimental results, all of which represented dorsoventral reaction in the collar, in terms of a gradient of growth-rates in the regenerating germ. This interpretation was based upon the discovery by Juhn and Gustavson (1930) that the threshold of reaction in feathers of different tracts is proportional to their rates of growth. Juhn, Faulkner, and Gustavson (1931) later showed that the reaction thresholds of feathers within certain contour tracts are likewise proportional to rates of growth during regeneration. In view of these findings, Lillie and Juhn concluded that

"it is logical, and indeed necessary, to hold that this principle applies to the parts of a feather as well as to different feathers." Fraps and Juhn (1936) questioned certain data presented by Lillie and Juhn on the growth of barbs; their results do not, however, disprove the existence of small but significant axial rate differentials, nor do they take into account growth differentials in other than the axial dimension.

Assuming that the concentration of hormone required to effect a given reaction is always proportional to rate of growth in the barb primordia, the three orders of pigmentation described above would require the postulation of three fundamentally different growth gradients in different collar limbs. Suppose, on the basis of this assumed proportionality, that we read "growth-rates" instead of "concentrations" in Figure 4. The collar of the breast germ would show gradually increasing growth-rates in dorsoventral order (curve *I*), as was postulated by Lillie and Juhn. The medial (or solidly black) vane-half of secondaries would require the assumption of increasing growth-rates in ventrodorsal order (curve *II*), and the lateral vane-half of the same feathers calls for a curve of growth-rates (curve *III*) with minimal values at an intermediate position, *h*, and increasing rates toward ventral and dorsal limits of the collar.

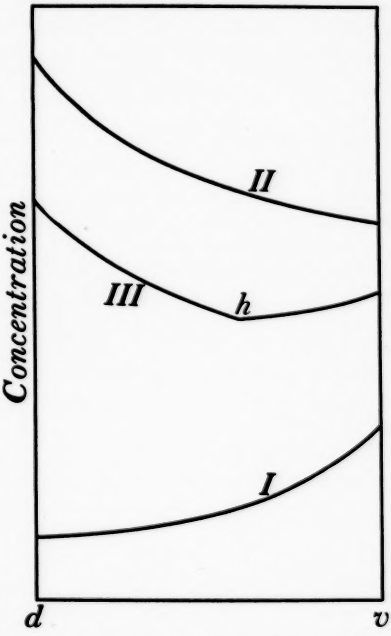


FIG. 4.—Relation between pigmentation loci in the collar (abscissas) and order of reaction to increasing hormone concentrations (ordinates) in different collar limbs. Note that abscissas are ranged in *dorsoventral order*. Curve *I:* breast or saddle germ collar. Curve *II:* the collar from medial vane-half of a secondary, showing the reverse relation of curve *I*. Curve *III:* collar from lateral vane-half of a secondary, combining the relations of curve *I* (*h* to ventral collar limit) and curve *II* (*h* to dorsal collar limit).

It seems necessary to suppose that the primary differentials associated with the organization, growth, and development of feather germs are fundamentally alike in all cases, however much germs may differ in scale of organization or in details. The "organizer" properties of the ventral triangle (Lillie and Juhn, 1937) and its field characteristics in certain feathers (Fraps, 1938), the invariable seriation of barb primordia, the increasing physiological age of primordia with ventrodorsal progression through the collar, and the general similarity in conformation of reactive centers for barb bases, for barbulation, and for pigmentation in different germs—all serve to enforce this view. But it is equally certain that the scale of organization of different germs (as breast and secondaries) together with rates of growth and differentiation vary over a relatively great range.

Given a system of primary, determinative gradient relations in the collar of feather germs generally (e.g., growth gradients), it follows that secondary processes dependent upon these primary differentials must also be subject to gradient differentials. We may safely assume that these secondary graded processes, or gradient functions, change by

differing degrees with change in absolute intensity levels of the primary gradient processes. Two such derivative or secondary gradients will vary also, then, with respect to each other at different absolute levels in the primary gradient system. Wherever two processes (rates, reaction potentials, etc.) are subject to graded intensities in the same or different collar limbs, but differ *with respect to each other* with differing values in an underlying gradient process, they may be denoted differential gradient functions. If, finally, we suppose that certain critical or limiting reactions determining alternative pigmentation processes are themselves differential gradient functions as thus defined, the several pigmentation orders described above can be accounted for satisfactorily without postulating the "reversal" of primary developmental gradients (such as the distribution of growth-rates). Application of this concept to the observed data follows.

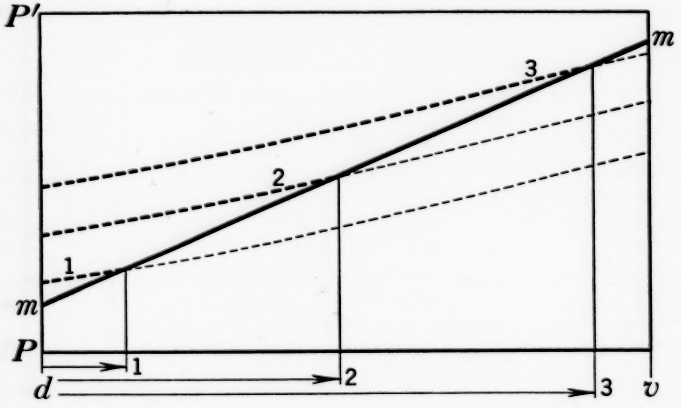


FIG. 5.—Differential gradient functions in the dorsoventral order of imposed pigmentation. Abscissas represent uniformly increasing primary gradient values in the dorsoventral order (from *d* to *v*). Ordinates represent limiting reaction potentials, increasing from *P* to *P'*. Curve *mm* indicates reaction potentials for melanization (in the breast feather). Curve 1: reaction potentials for imposed pigmentation at low concentration of female hormone, displacing melanization reactions from *d* to level 1 on abscissas. Curves 2 and 3: gradients of reaction potentials for imposed pigmentation at two higher concentrations of female hormone, extending the imposed processes to collar levels 2 and 3, respectively.

INTERPRETATION OF THE PIGMENTATION ORDERS

a) *The dorsoventral order.*—The assumption of a direct proportionality between hormone threshold and rate of cell division made by Lillie and Juhn accounted adequately for the dorsoventral extension of imposed pigmentation with rising hormone concentrations in breast and saddle feathers. This interpretation, however, tells us nothing about relations obtaining between the primary gradient (growth-rates) and the conditions determining the partially or completely displaced pigmentation—black in breast feathers, buff in saddle feathers. We accordingly consider this case first.

The differential gradient relations required by the dorsoventral order of pigmentation (breast feathers as example) are given in Figure 5. Let the axis of abscissas, *dv*, represent primary gradient values (as growth-rates), increasing uniformly from *d* to *v*. Let ordi-

nates, PP', represent potentials (intensity, rate, or other values) for the critical, limiting reaction entering into alternative pigmentation processes. If this critical reaction potential for black (in the breast feather) is taken as a direct function of the primary gradient, it may be represented in Figure 5 by the solid line, *mm*. The indicated proportionality between the primary gradient values (abscissas) and the limiting reaction potential for melanization does not necessarily mean that rate of pigmentation, or quantity of pigmentation deposited, increases from dorsal to ventral limits of the primary gradient. This may or may not be true. In the present connection, however, the curve, *mm*, means simply that the reaction potential of the limiting process involved in melanization increases with increasing values of the primary gradient.

When female hormone effects, whether directly or indirectly, an alternative pigmentation formation (salmon instead of black in the breast feather), it is necessary to assume again some proportionality between primary gradient values and potentials of the limiting or critical reaction in the imposed pigmentation processes. This proportionality cannot be identical with that for melanization in the breast feather, since this would mean the simultaneous displacement of black from the entire collar limb at minimal concentrations of hormone inducing any formation of salmon. Since the imposed pigmentation (salmon) appears at minimal concentrations of hormone in dorsally limited regions of the collar, the curve of limiting reaction potentials must have a lesser slope than does the corresponding curve for melanization; only such a proportionality with the primary gradient differentials can have higher potentials in dorsalmost collar regions with minimal hormone concentrations. The required gradient function is represented by curve 1 of Figure 5. Reaction potentials for imposed (salmon) pigmentation processes are higher than are potentials for melanization from *d* to collar level 1, while from level 1 to *v* the relation between the two gradients is reversed. With increased hormone concentration all reaction potentials for salmon are increased (not necessarily uniformly) and we obtain curve 2: all collar loci between *d* and level 2 now react for salmon pigmentation, all loci from collar level 2 to *v* for melanization. Curve 3 represents the gradient of reaction potentials with a concentration of hormone just below the concentration required for complete displacement of melanization processes from the entire collar limb, only ventralmost regions (collar level 3 to *v*, Fig. 5) now having the higher reaction potentials requisite for maintenance of melanization.

Actually, of course, we do not know the absolute reaction potentials for the gradient function represented either by *mm* or by the three imposed curves associated with differing hormone concentrations. We do know that alternative reactions occur dorsally and ventrally from precise loci in the primary gradient (collar levels 1, 2, and 3) at which limiting reaction potentials are equivalent in the secondary gradient functions, and we know also that increasing hormone concentrations must extend the imposed pigmentation processes in dorsoventral order. These relations are satisfied by differential gradient functions of the *order* given in Figure 5.

We have tacitly assumed in the foregoing treatment that the primary gradient values (abscissas of Fig. 5) and the secondary gradient function, *mm*, remain unchanged with differing hormone concentrations. This is probably not the case in breast feathers, and almost certainly not so in the saddle germ under the action of thyroxin. Nevertheless, as long as the general relationship between potentials for alternative pigmentation reaction is maintained in the form represented in Figure 5, the experimentally determined conditions for dorsoventral pigmentation are satisfied.

b) *The ventrodorsal order.*—The ventrodorsal order of pigmentation proceeds from ventral to dorsal limits of the collar with increasing hormone concentration, just the reverse of pigmentation in the breast germ. We consider here only the medial (solidly black) vane-half of the secondaries.

The required relation between secondary gradients representing reaction potentials is given in Figure 6. Abscissas indicate uniformly increasing values in the primary gradient from *d* to *v;* this is the same order of primary gradient differentials followed in Figure 5, but absolute values are presumably much higher in the present case in view of the relatively high growth-rates of regenerating secondaries. Reaction potentials are read as ordinates. Again we assume a proportionality between the secondary gradient function representing limiting reaction potentials for melanization and primary gradient

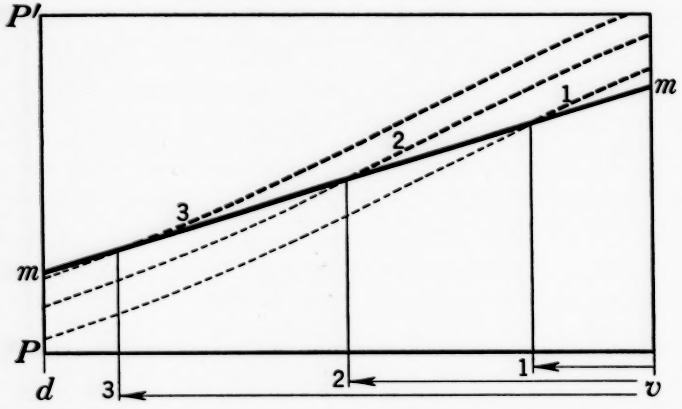


FIG. 6.—Differential gradient functions in the ventrodorsal order of pigmentation. Abscissas: uniformly increasing primary gradient values in dorsoventral order (from *d* to *v*), *the same order* represented in Figure 5, but absolute values understood to be higher. Ordinates: limiting reaction potentials, increasing from *P* to *P'*. Curve *mm:* reaction potentials for melanization. Curve 1: reaction potentials for imposed pigmentation at relatively low concentration of female hormone: melanization is displaced from *v* to collar level 1 (reverse of dorsoventral order, Fig. 5). Curves 2 and 3: gradients of reaction potentials for imposed pigmentation at two higher concentrations of female hormone, displacing melanization processes from ventral collar limit to levels 2 and 3, respectively.

values, giving us the curve *mm* of Figure 6. A relatively low concentration of female hormone induces reaction in ventral collar limits, and we accordingly draw curve 1 *more steeply* than the curve *mm*. This simply means that the limiting or critical potentials for the hormone-induced reaction (stippling) increases relatively more rapidly than do the potentials for melanization with increasing values (reading from *d* to *v*) in the primary gradient. Given this particular differential proportionality between the secondary gradients, the induced pigmentation must appear at the ventral limit of the germ with minimal hormone concentrations, and it will be extended through the collar in ventrodorsal order with increasing concentrations. Thus, in Figure 6 a relatively low hormone concentration establishes the reaction potentials of curve 1, with stippling over the range *v* to collar level 1. An intermediate concentration gives us curve 2, with stippling from *v*

to collar level 2; and a relatively high concentration further raises the gradient of reaction potentials for induced pigmentation to the level of curve 3, with stippling imposed over the range *v* to collar level 3. Each of the three curves represents, of course, the stabilized gradient of reaction potentials established by a given concentration of hormone; with gradually increasing concentrations a continuous series of such gradients are imposed in the collar of the germ.

A comparison of Figures 5 and 6 makes it clear that, in terms of differential gradient functions, dorsoventral (Fig. 5) or ventrodorsal (Fig. 6) pigmentation orders represent only differing proportionalities, at differing absolute values in the primary gradient, in the secondary gradients of reaction potentials determining alternative pigmentation processes. This is best seen by comparing the relative slope of curve 1 in each figure with the curve for melanization, *mm*, which it intersects: the relative slopes of these intersecting curves represent the relative proportionalities between alternative reaction potentials over differing ranges of absolute values in the primary gradients (abscissas of Figs. 5 and 6).

c) The complex order.—"Complex" pigmentation orders are incident at a median point in the collar (*h* of Fig. 1) with minimal hormone concentration and proceed toward ventral and dorsal collar limits with increasing concentrations. The certain examples occur in the brown-black vane-halves of the secondaries, with incidence of the imposed pigmentation at the boundary between brown and black reactions. We must, accordingly, begin with some relationship beween reaction potentials for the alternative pigmentation processes of the normal feather. The most likely interpretation is that reaction potentials for these alternatives represent differential gradient functions in exactly the sense that experimentally imposed and "normal" reaction potentials have already been related.

In Figure 7 abscissas indicate increasing values in the primary gradient from dorsal to ventral collar limits; ordinates represent reaction potentials. Let the curve *mm* represent the gradient of reaction potentials for melanization, and *rr* the gradient of reaction potentials for brown pigmentation. Melanization will then occur from the dorsal limit of the collar, *d*, to the intermediate position, *h;* and from *h* to the ventral collar limit, brown pigmentation processes are completed (cf. Fig. 1). The fact that the boundary between brown and black approaches the shaft in the order of increasing growth-rates through the sequence of secondaries suggests certainly that some such purely quantitative relation determines alternative reactions.

If now we suppose the gradient of reaction potentials for the hormone-induced pigmentation processes (stippling) to be steeper than that for melanization, but less steep than that for formation of brown, the initial point of imposed pigmentation must occur at the boundary between brown and black reactions. This condition is satisfied in Figure 7 by any straight line taking its origin between *m* and *r* and passing through *o*. The experimentally determined patterns suggest, however, that the concentration of hormone required for modification of the brown-producing collar regions is much less than that required for equivalent modification in the black-producing regions. We accordingly draw *ss* through *o* to make the angle *sor* relatively small and the angle *som* relatively great. In other words, we represent the gradient of reaction potentials for induced pigmentation as differing relatively little from the gradient of reaction potentials for brown pigmentation. If the two gradients of reaction potentials (for induced and for brown pigmentation) represented identical functions of the primary gradient, brown

should be displaced simultaneously from collar level *h* to the ventral limit of the collar with no appreciable displacement from *h* toward the dorsal limit (*rr* and *ss* of Fig. 7 would, in this instance, be identical).

A second gradient of reaction potentials, established by a higher concentration of hormone, is indicated by curve 2 of Figure 7. Induced pigmentation processes have completely displaced brown; and the order of displacement is from collar level *h* toward *v*, the ventral limit of the collar (dorso-ventral order). Melanization processes are displaced, however, over only the limited range *h* to collar level 2, and in ventrodorsal order. With a considerably higher concentration of hormone we obtain curve 3, leaving

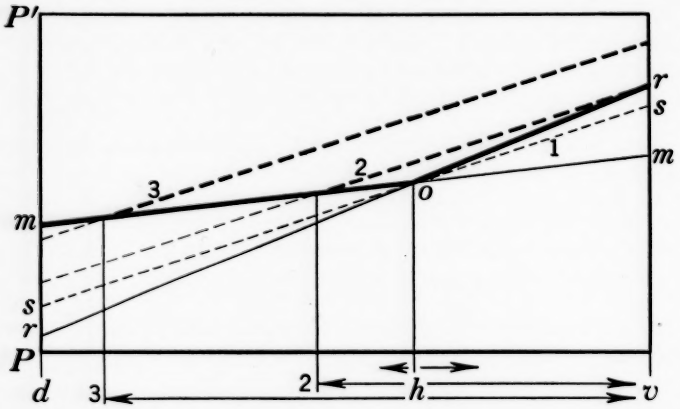


FIG. 7.—Differential gradient functions in the "complex" order of pigmentation. Abscissas: primary gradient values in increasing dorsoventral order (from *d* to *v*). Ordinates: limiting reaction potentials, increasing from *P* to *P'*. Curve *mm:* reaction potentials for melanization in the normal feather. Curve *rr:* reaction potentials for brown in the normal feather. These two gradients of the normal reaction potentials, intersecting at *o*, refer the boundary of normal brown-black pigmentation to the collar level *h*, corresponding to *h* of Figure 1. Curve *ss:* gradient of reaction potentials for minimal concentration of hormone effecting imposed reaction (stipple). Curves 2 and 3: gradients of reaction potentials for higher concentrations of hormone. Curve 2 completely displaces processes for brown pigmentation in dorsoventral order (from *h* to *v*), and displaces melanization in ventrodorsal order (from *h* to level 2). Curve 3 further displaces melanization in ventrodorsal order to collar level 3.

only a dorsally limited collar region (level 3 to *d*, Fig. 7) in which melanization continues. Still higher concentrations of course displace melanization from this region also, producing reaction for stippling through the entire extent of the collar.

The plumage of the Brown Leghorn male or capon exhibits a wide range of pigmentation combinations, only one of which—the brown-black combination in the secondaries—has just been considered in terms of differential gradient functions. A simple and wholly theoretical application of differential functions over a relatively great range of values in an assumed primary gradient will at least suggest a basis for the observed diversity of pigmentation combinations generally.

Abscissas of Figure 8 indicate increasing values (from *G* to *G'*) in an assumed primary

gradient; all values found in an array of collar limbs are included on this single axis of abscissas. Reaction potentials are read as ordinates. Let the curve *mm* represent the gradient of reaction potentials for melanization, and curve *rr* the gradient of reaction potentials for an alternative pigmentation—say red in order to distinguish this theoretical case from the Brown Leghorn pattern considered above. We assume that the general physiological conditions of the bird remain uniform, which is to say that the two gradients for alternative reaction potentials are stable for the case under discussion.

In terms of Figure 8, then, all collar limbs with primary gradient values falling between *G* and *h'*, and *h* and *h''*, must produce red pigmentation; all collar limbs with pri-

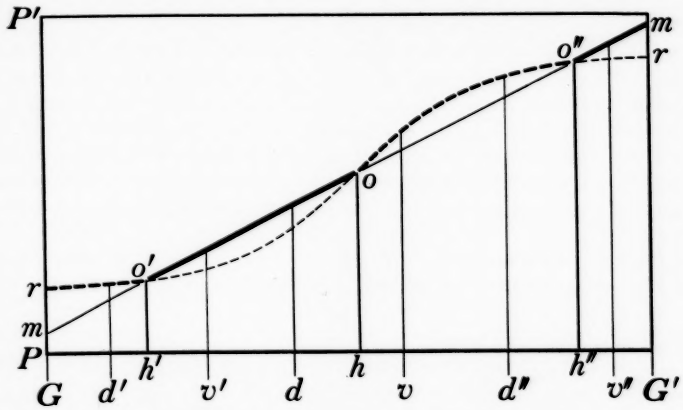


FIG. 8.—Theoretical relation between continuous differential gradient functions required for "reverse" and complex pigmentation orders in different feathers. Abscissas: increasing values in primary differentials (from *G* to *G'*) for an array of collar limbs. Ordinates: limiting reaction potentials. Curve *mm:* reaction potentials for melanization. Curve *rr:* reaction potentials for red pigmentation. All collar limbs with primary gradient values falling between *G* and *h'*, and *h* and *h''*, can produce only red pigmentation; all collar limbs with primary gradient values falling between *h'* and *h*, and *h''* and *G*, can react only for melanization. Collar limbs including *h'*, *h*, or *h''*, corresponding to equivalent reaction potentials *o'*, *o* and *o''*, will exhibit complex pigmentation orders. Collar limbs *d'v'*, and *d''v'':* red dorsally, black ventrally. Collar limb *dv:* black dorsally, red ventrally.

mary gradient values falling between *h'* and *h*, or *h''* and *G'*, can react only for melanization. Any collar limb including the values *h'*, *h*, or *h''*, corresponding to equivalent reaction potentials *o'*, *o*, or *o''*, will produce complex pigmentation patterns. Collar limbs *d'v'* and *d''v''* must each react for red pigmentation from a median collar level, *h'* or *h''*, to the dorsal limits of the collars, *d'* and *d''*. From the same medial point, *h'* or *h''*, to the ventral collar limits, *v'* and *v''*, melinization takes place. Thus, at two extremes of primary gradient values we should obtain feathers with an identical complex pattern, a red central region with black marginal areas. The collar limits *dv* (Fig. 7) will also produce a complex pigmentation pattern, but in this case the feather will have a black central region with red forming marginally, as brown does in the lateral vane-half of secondaries in the Brown Leghorn (Fig. 3*A*).

4. DISCUSSION

The obvious limitation in the foregoing interpretations of the several experimentally induced pigmentation orders is that we cannot define with precision the limiting reaction potentials treated as differential gradient functions. From this eventual point of view the representations given in this paper are formal, as they must remain to varying degree until our knowledge of physical and chemical processes constituting the chain of events from initial genic action to final deposition of pigment has been greatly extended. In the meantime there is at hand the considerable body of physiologically significant relationships that are satisfactorily accounted for in principle. The identification and quantitative formulation of the postulated limiting reaction potentials becomes a main problem for future investigation.

From a genetic point of view, however, the postulation of an independent gradient of critical reaction potentials for each pigmentation process is by no means an arbitrary assumption. In sexually dimorphic plumages, at least, the colors that can be produced by hormones are genetically represented in all cells of reactive follicles (Danforth, 1933). The same is presumably true of those pigmentation reactions that are independent of hormone action or below the threshold of hormone effect. Diversity of genetic resultants appearing as pigmentation alternatives must then be due to extra-genic differentials, and in the present paper these have been described as differential functions of primary gradients. This assumes, of course, differing reaction chains for differing pigmentation products. The existence of such differing reaction chains must certainly be granted wherever differing genic action is involved, for each gene or genic complex potentially capable of producing an independent or differentiated resultant must do so through a sequence of reactions differing in some degree from the series of reactions that can be attributed to any other gene or genic complex.

Differential gradient functions represent the relationship between gradients of limiting or critical reactions in two such independently initiated reaction series. It is not necessary to assume that the action of a gene initiating a reaction chain is itself a function of primary gradient differentials; this may or may not be the case. It is only necessary to suppose that critical reactions in the series become subject to the primary gradients before completion of the pigmentation process. This may occur at any time after initial genic action, and must occur whenever the reaction chain involves processes (or substances) which are directly or indirectly functions (as distributions of rates, substances, etc.) of primary gradient differentials. And, remembering the "discrete" genic initiation of alternative reaction series, it seems necessary to hold that component reactions in each chain cannot ordinarily be identical functions of the primary gradients but will, on the contrary, exhibit a differential relationship of the order represented in the several figures previously considered.

The *order* of pigmentation of feathers under varying hormone concentrations has been studied quantitatively in only a single bird, the Brown Leghorn, and in any detail for only certain of the feather tracts. The results obtained with this bird demonstrate beyond all doubt the fundamental role of purely quantitative differentials in the localization of pigmentation reactions in the individual feather. This is true altogether apart from the interpretation placed upon the observed quantitative relationships. The further analysis of these differentials and the testing of hypotheses designed to interpret them call for application of the quantitative procedures of Lillie and Juhn to plumages

of differing genetic compositions. A comprehensive investigation of differing genetic compositions by these methods might reasonably be expected to throw some light on the mechanism of genic action as well as to clarify the nature of the differentials through which the localization of resultants is effected.

5. SUMMARY

1. Three orders of pigmentation induced in feathers of the Brown Leghorn male or capon with increasing hormone concentration are described. These are, in terms of loci in the collar of the developing germ: (*a*) the dorsoventral order previously described in detail by Lillie and Juhn; (*b*) the ventrodorsal order, which is completely the reverse of (*a*); and (*c*) a complex order, proceeding from a medial point in the collar toward both ventral and dorsal collar limits.

2. These orders of pigmentation are interpreted in terms of differential gradient functions, that is, in terms of secondary gradients in the limiting reaction potentials for alternative pigmentation processes. Each of these secondary gradients, or gradient functions, is assumed to vary by differing proportionalities with a given change in primary gradient differentials, which are assumed to remain always the same in direction although varying in absolute values in collar limbs of different germs.

LITERATURE CITED

DANFORTH, C. H. 1933. Genetic factors in the response of feather follicles to thyroxin and theelin. Jour. Exper. Zoöl., **65**:183–97.

FRAPS, RICHARD M. 1938. Effects of thyroxin and female hormone on one phase of saddle feather development. Proc. Soc. Exper. Biol. and Med., in press.

FRAPS, RICHARD M., and JUHN, MARY. 1936. Developmental analysis in plumage. II. Plumage configurations and the mechanism of feather development. Physiol. Zoöl., **9**:319–77.

GREITE, WALTER. 1934. Die Strukturbildung der Vogelfeder und ihre Pigmentierung durch Melanine. Zeitschr. f. wiss. Zool., **145**:283–336.

HOSKER, ANNE. 1934. Bilateral gyandromorphism in feathers. Nature, **133**:382–83.

———. 1936. Studies on the epidermal structures of birds. Phil Trans. Roy. Soc. London, B, **226**:143–88.

JUHN, MARY; FAULKNER, G. H.; and GUSTAVSON, R. G. 1931. The correlation of rates of growth and hormone threshold in the feathers of fowls. Jour. Exper. Zoöl., **58**:69–111.

JUHN, MARY, and GUSTAVSON, R. G. 1930. The production of female genital subsidiary characters and plumage sex characters by injection of human placental hormone in fowls. *Ibid.*, **56**:31–61.

LILLIE, FRANK R. 1932. The physiology of feather pattern. Wilson Bull., **44**:193–211.

LILLIE, FRANK R., and JUHN, MARY. 1932. The physiology of development of feathers. I. Growth-rate and pattern in the individual feather. Physiol. Zoöl., **5**:124–84.

———. 1937. The origin of the after-feather in fowl: a process of twinning. Science, **86**:38–39.

SINGLE AND COMBINED EFFECTS OF CYANIDE AND METHYLENE BLUE ON RECONSTITUTION IN EUPLANARIA DOROTOCEPHALA[1]

(Eight figures)

OLIN RULON
University of Chicago and Wayne University

IT HAS long been known that cyanide exerts its chief effects on living protoplasm by inhibiting respiratory metabolism. It is also known that cyanide may retard cell division and growth. Certain experiments by various workers (Hug, 1932; Brooks, 1932, 1933; Bodine and Boell, 1936, 1937; and others) have shown that methylene blue, in certain concentrations, will antagonize the poisonous effects of cyanide and restore the normal respiration in whole adult animals and in developing embryos. In contrast with the effects of cyanide, methylene blue has been found, in certain cases, to increase the rate of respiratory metabolism above the normal.

Child (1916) found that it was possible to alter and control the character of the head formed and the frequency of head formation in pieces of *Euplanaria* through the use of low concentrations of KCN. It was apparent from his experiments that cyanide not only inhibited the growth of new tissue but exerted inhibitory effects on other factors related to the reconstitutional process. With this work in mind the present research was undertaken in an attempt to determine additional effects of cyanide, the effects of methylene blue, and the combined effects of the two on reconstitutional development.

MATERIAL AND METHODS

The material used in this investigation was the common flatworm of the Chicago region, *Euplanaria dorotocephala*. The animals were usually kept in the laboratory for several weeks after collection and were not fed for approximately 2 weeks before the experiments were begun. Animals, 14–16 and 15–17 mm. in length, were used; and of these, only pieces from the anterior zooid between the mouth and head were tested. The pieces were approximately one-eighth the length of the entire animal, exclusive of the head, and were labeled from anterior to posterior: *A*, *B*, *C*, and *D* (Fig 1). In all experiments lots of 50 pieces from each region were used. These lots were obtained by sectioning 65 animals and selecting 50 pieces of most nearly the same size from each region. The pieces were then placed in the test solutions in 1-liter flasks for varying intervals of time after section. All experiments in testing the effects of methylene blue and cyanide on reconstitution were controlled by the reconstitution of similar pieces in well water.

Animals reconstituting in the test and control solutions were kept in the dark at room temperatures for the entire reconstitutional period. After the reconstitutional period, which lasted approximately 2 weeks, the animals were removed from the flasks and examined with a binocular microscope to determine the type of head and other reconstitutional phenomena. The different head types have been classified by Child (1911*a*, 1911*b*, 1911*c*, 1912, 1914*a*, 1914*b*, 1916, 1920, 1924) on the degree of inhibition,

[1] Support for this work was provided from a grant by the Rockefeller Foundation in aid of research in the biological sciences at the University of Chicago.

and this classification is followed in this work. Briefly stated, the different types are: (1) *normal*—with triangular head, auricles at lateral margins, and two symmetrically placed eyes; (2) *teratophthalmic*—with triangular head and auricles in normal position but with eyes showing all degrees of approximation to the median plane from two distinct eyes with pigment connected up to complete cyclopia; (3) *teratomorphic*—with head more or less rounded in outline and with auricles more or less anterior and showing all degrees of approximation to the median plane up to a single median auricle and with a single, or apparently single, median eye; (4) *anophthalmic*—with head rudimentary, without eyes, and with or without a single median auricle; (5) *acephalic* with head completely absent.

Child and Watanabe (1935) have given a fairly reliable method for the evaluation of the approximate average degree of head inhibition of pieces from a particular level and of a given size. In this method each type of head is designated by an arbitrary number that corresponds roughly to the degree of inhibition. The arbitrary values of each type are as follows: normal, 100; teratophthalmic, 80; teratomorphic, 60; anophthalmic, 40; acephalic, 20. The head-frequency index (designated as "mean" by Child and Watanabe) is calculated for any particular lot of worms by multiplying the number of each head type by its arbitrary number and dividing the sum of all by the total number of living animals. It is easily seen that animals reconstituting from *A* levels, which usually develop almost 100 per cent normal heads, will have an index approaching 100, while animals reconstituting from *D* levels, which usually give a high percentage of strongly inhibited head types under control conditions, will have an index approaching 20.

Since many experiments in this laboratory by various workers have shown that a comparison of head-frequency indices of test and control experiments gives a reliable method for discerning the effects of various agents on reconstitution, graphs showing the indices plotted against body-level are used throughout this paper in the presentation of the data.

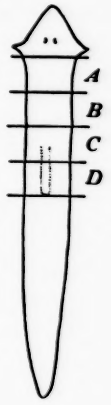


FIG. 1.—Levels *A*, *B*, *C*, and *D* used in all head-frequency experiments. Each section is approximately one-eighth the length of the animal, exclusive of the head. Head and region posterior to the mouth discarded.

EXPERIMENTAL DATA

1. *The effect of KCN on head frequency.*—Ten different experiments were performed on different stocks of worms in an effort to determine additional effects of cyanide on head frequency. Each experiment involved 50 test and 50 control pieces from each of the four different levels. In the tests the pieces were placed in M/100,000 KCN immediately after section, and kept in this solution for 72 hours. After this initial exposure to the cyanide the pieces were transferred to well water for the remainder of the experimental period. (Pieces left in this concentration of KCN for the entire reconstitutional period are so pigmented and inhibited that it is next to impossible to catalogue the different head types.)

In seven of the ten experiments testing the effect of cyanide on head frequency, the controls showed a relatively high head frequency, while the remaining three showed a

considerably lower head frequency. The differences in head frequency in different stocks have been dealt with in previous work (Rulon, 1936). Of the seven experiments with a high head frequency in the controls, the tests in every case (in pieces from *B*, *C*, and *D* levels) showed a lower head frequency. This is shown graphically in Figure 2. In the graph the reconstituted forms from the 350 control animals are averaged together to give curve *L*, while the 350 test animals are shown as curve *M*.[2]

In the three experiments in which the controls showed a low head frequency, the tests behaved somewhat differently. Pieces from *A*, *B*, and *C* levels treated with cyanide give

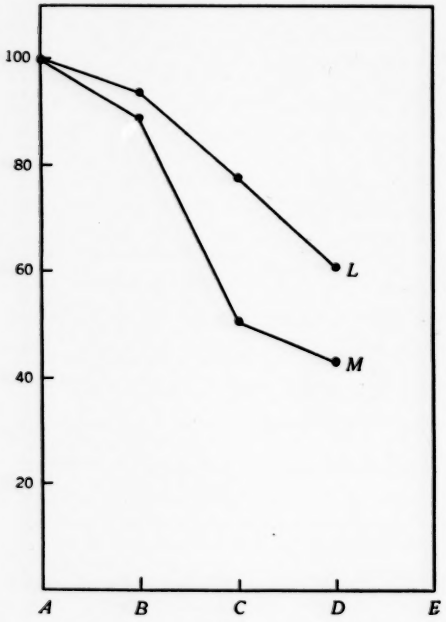


FIG. 2

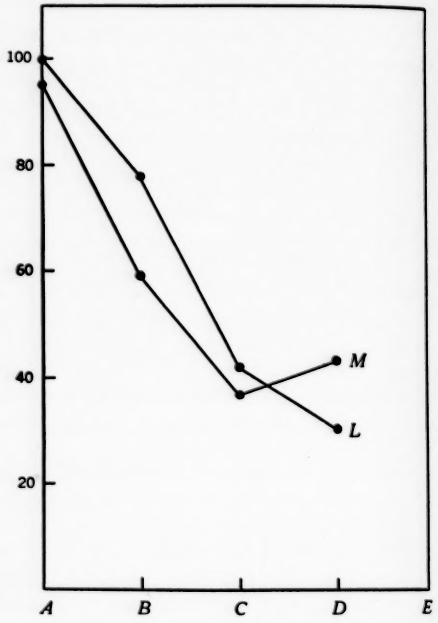


FIG. 3

FIG. 2.—Combined data of seven experiments showing the effects of cyanide on head frequency of animals having a high head frequency in the controls. *L* = control. *M* = test.

FIG. 3.—Combined data of three experiments showing the effects of cyanide on head frequency of animals having a low head frequency in the controls. *L* = control. *M* = test.

a lower head frequency than the controls, while pieces from *D* levels show a distinctly higher head frequency. This is shown graphically in Figure 3, in which the 150 test animals are shown by curve *M* and the control animals by curve *L*.

2. *The effect of methylene blue on head frequency.*—In order to determine a suitable concentration, experiments were run in which intact animals were submitted to various solutions of the dye for different intervals of time. It was found, in general, that intact

[2] All graphs are plotted with the head-frequency indices as ordinates and body levels as abscissas.

animals (14–16 mm. in length) could live for 2–3 days in 1/100,000 and for 5–7 days in 1/200,000 solution. These animals became heavily stained, inactive, and strongly insensitive before cytolysis finally set in. Short pieces (one-fourths of the anterior zooid) were more susceptible to these concentrations; and, although they could live and reconstitute in 1/500,000, the best results were obtained by allowing the pieces to stay in 1/200,000–1/300,000 methylene-blue solution for 72 hours and then returning them to well water for the remainder of the reconstitutional period. At the end of 72 hours in the dye the pieces are deeply stained and are relatively insensitive to external stimuli.

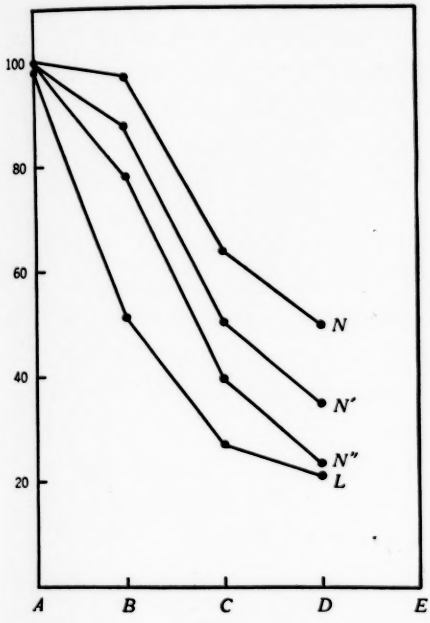


FIG. 4

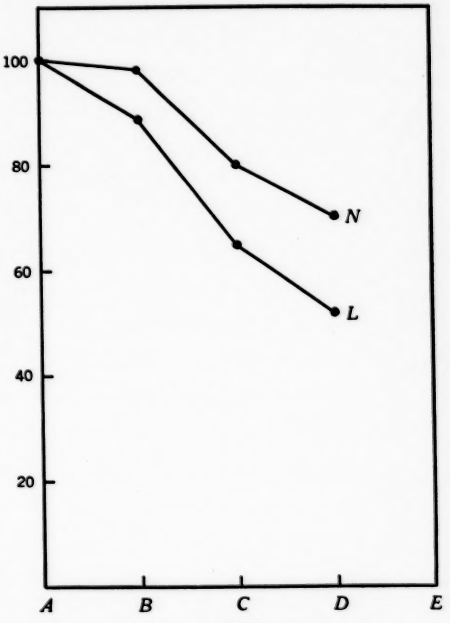


FIG. 5

FIG. 4.—Data of one experiment showing the effects of different concentrations of methylene blue on head frequency. L = control. N = 1/200,000 methylene blue for 60 hours. N' = 1/500,000 methylene blue for entire reconstitutional period. N'' = 1/1,000,000 methylene blue for entire reconstitutional period.

FIG. 5.—Combined data of nine experiments showing the effects of 72 hours' exposure to 1/200,000–1/300,000 methylene blue on head frequency. L = control. N = test.

They retain considerable of their blue stain after return to water, and even 2 weeks later the gut cells may contain many blue granules.

A comparison of long-time exposure to weak solutions and short-time exposure to stronger solutions of methylene blue is shown graphically in Figure 4. Fifty pieces were used for each level in the control and each test. Curve L represents the head frequency of the control in well water. The head frequency of animals reconstituting for the first 60 hours in 1/200,000 methylene blue and for the remainder of the period in well water

is shown by curve *N*. Pieces reconstituting for the entire period in 1/500,000 methylene blue are shown by curve *N'* and for the entire period in 1/1,000,000 by curve *N''*. These experiments show quite definitely that methylene blue increases the head frequency over a range of concentrations but that short-time exposure to higher concentrations is more effective.

Nine experiments were performed in which the tests were submitted to 1/200,000–1/300,000 methylene blue for the first 72 hours after section. In every case pieces from *B*, *C*, and *D* levels treated with methylene blue gave a higher head frequency than the

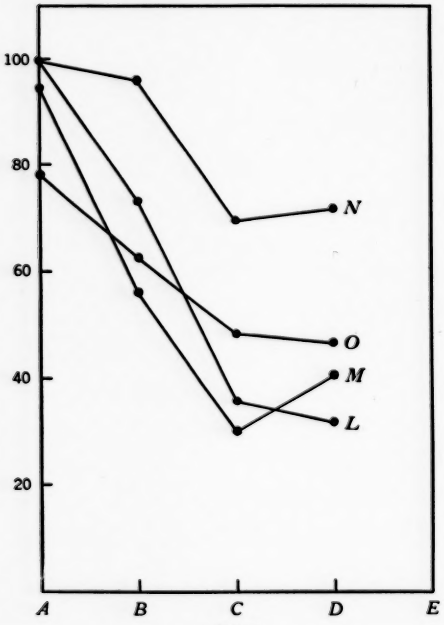


FIG. 6

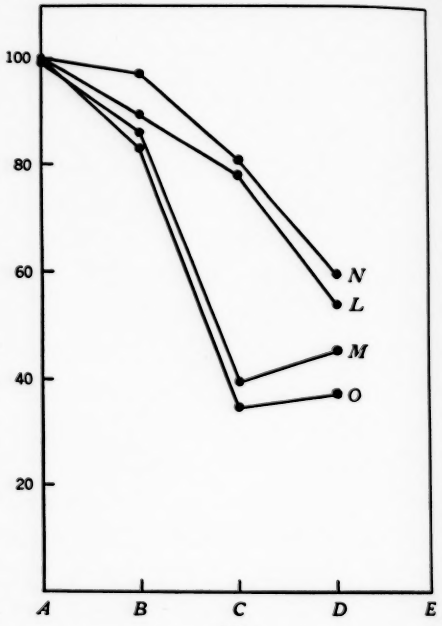


FIG. 7

FIG. 6.—Combined data of two experiments showing the single and combined effects of 1/300,000 methylene blue and M/100,000 KCN on head frequency of animals with a low-control head frequency after 72 hours' exposure. *L*=control. *M*=cyanide. *N*=methylene blue. *O*=combined solution of cyanide and methylene blue.

FIG. 7.—Combined data of two experiments showing the single and combined effects of 1/300,000 methylene blue and M/100,000 KCN on head frequency of animals with a high-control head frequency after 72 hours' exposure. *L*=control. *M*=cyanide. *N*=methylene blue. *O*=combined solution of cyanide and methylene blue.

controls for similar levels. In general, pieces from lots with a low head frequency showed a greater increase in head frequency when treated with methylene blue than did pieces from lots that gave a high head frequency in the controls. In *A* pieces no effects of the methylene blue could be detected in head frequency, since pieces from this level in both the tests and controls reconstituted nearly 100 per cent normal heads. The head-

frequency indices of these nine experiments (450 control and 450 test animals) are averaged and shown graphically in Figure 5. Curve *L* represents the average head frequency of the controls; and curve *N*, the tests.

It has been noted that many of the animals reconstituting after exposure to methylene blue appear somewhat swollen and in many cases considerably more pigmented than in the controls. These phenomena are of more common occurrence in pieces from the more anterior levels.

3. *The combined effect of methylene blue and KCN on head frequency.*—Two series of experiments were performed in which stocks showing a low head frequency in the con-

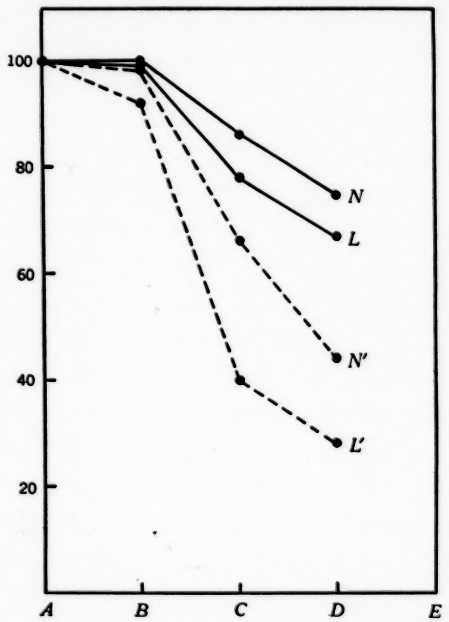


FIG. 8.—Data of one experiment showing the effects on head frequency by 6 days' exposure of intact animals to M/100,000 KCN prior to section, and the recovery in well water and methylene blue after section. Animals treated with cyanide shown by broken lines. *L* = untreated control. *L'* = cyanide-treated animals reconstituted in well water. *N* = untreated methylene blue. *N'* = cyanide-treated animals exposed to methylene blue after section.

trols were used (Fig. 6, curve *L*). Such animals gave results similar to those shown in Figure 3 when treated with M/100,000 KCN for 72 hours after section (Fig. 6, curve *M*). It will be noted that the head frequency of animals from *A*, *B*, and *C* levels is below, and of *D* level above, that of the controls. Similar pieces treated with 1/300,000 methylene blue show a tremendous increase in head frequency at *B*, *C*, and *D* levels (Fig. 6, curve *N*). Pieces submitted to the combined solution (M/100,000 KCN; 1/300,000 methylene blue) for 72 hours after section gave very interesting head frequencies (Fig. 6, curve *O*). It is to be noted that *A* pieces show a definitely lowered head frequency when treated

with this combination. The head frequency of animals from *B* pieces is higher than that of animals which have been treated with KCN alone but lower than the controls. The head frequencies of *C* and *D* pieces are distinctly higher than the controls or those treated with KCN.

Two series of experiments were performed in which the stocks showed a relatively high-control head frequency (Fig. 7, curve *L*). These animals gave results similar to those shown in Figure 2 when treated for 72 hours with M/100,000 KCN, in that the head frequencies of pieces from *B*, *C*, and *D* levels are below the controls (Fig. 7, curve *M*). Methylene blue, as in all other experiments, increased the head frequency of pieces from *B*, *C*, and *D* levels. The combined solution (M/100,000 KCN; 1/300,000 methylene blue) gave results, however, that were considerably different than those from stocks which show a low head frequency in the controls. Pieces from *A* level were apparently unaffected, while *B*, *C*, and *D* pieces gave head frequencies *below* those of cyanide alone (Fig. 7, curve *O*).

One series of experiments was performed in which a lot of 15–17 mm. worms, showing a very high head frequency in the controls, was placed in M/100,000 KCN for 6 days prior to section. After this initial exposure of intact animals to the cyanide solution, they were sectioned, as in the preceding experiments (anterior zooid cut in fourths). The pieces from the different levels were then allowed to reconstitute (1) in well water and (2) in 1/200,000 methylene blue for 72 hours and then in well water. Equal numbers (50 worms for each test and control) of the same size and from the same stock, but without the cyanide treatment, were allowed to reconstitute in well water and 1/200,000 methylene blue (72 hours). The head frequency data of this experiment are shown graphically in Figure 8. The animals treated with cyanide prior to section are represented by dotted lines, while those untreated with this agent are shown by solid lines. It is readily seen that animals treated with cyanide before section and then allowed to reconstitute in well water (curve *L'*) show a distinctly lower head frequency at *B*, *C*, and *D* levels than untreated animals in well water (curve *L*). Likewise, cyanide-treated animals reconstituting for 72 hours in methylene blue show a lower head frequency (curve *N'*) than untreated animals with the same exposure to methylene blue (curve *N*). The important point to consider in this experiment, however, is the fact that methylene blue increases the head frequency of cyanide-treated worms over the treated worms which have reconstituted in well water. In other words, methylene blue appears to partially antagonize the effects of an initial exposure to cyanide.

DISCUSSION

It has been pointed out in previous work that, under normal conditions, at least two different factors determine whether or not short transverse pieces will reconstitute a head. The first of these factors has to do with the anteroposterior gradient of the intact animal. When a single section is made in *Euplanaria* at any level, a new head will be reconstituted. In other words, if the anterior portion of the animal is removed, a new head will grow on the posterior portion. The rate of reconstitution of the new head is dependent upon the level at which the section is made (Watanabe, 1935*a*). When the section is made at the anterior level of the first zooid, the rate is more rapid than when the section is made at a more posterior level of the same zooid. Such experiments show an anteroposterior gradient in rate of head development.

The second factor affecting head development is of a different nature. When short transverse pieces from the different levels reconstitute, it has been found that pieces of the same size but from different levels will not reconstitute in a like manner. Pieces, one-eighth the length of the intact animal, from a region just posterior to the head, will usually reconstitute almost 100 per cent normal heads; but pieces of the same length from more posterior levels of the same zooid (*D* pieces) usually reconstitute a high percentage of headless forms or forms with inhibited heads. It has been pointed out in previous work that the inhibition of heads in short transverse pieces is due to some factor or factors which arise at the posterior cut surface of the piece. This head-inhibiting factor may last for several days during the early stages of tail formation. Child and Watanabe (1935) and Watanabe (1935*b*) have shown that the head-inhibiting factor is probably conducted from its posterior point of origin to the head-forming region by way of the ventral nerve cords. If the head-inhibiting factor can be blocked or eliminated, or the head-forming cells near the anterior cut surface be given a start in the reconstitutional process (i.e., by delaying posterior section by 12 hours or more), animals with normal heads may be caused to reconstitute from short pieces of any level.

Since the rate of head formation is highest at more anterior levels, it has been suggested that the reason short pieces from anterior levels reconstitute normal heads while pieces of the same size from more posterior levels do not is because the more active head-forming regions of the anterior pieces may get out from under control of the posterior inhibiting factors, whereas the less active head-forming regions of posterior pieces are unable to do so. Of course, since we have no way for complete analysis, it may equally be true that the posterior head-inhibiting factor may be more active at the more posterior levels.

A large number of different agents have been used to increase the head frequency of short posterior pieces of *Euplanaria* by blocking, or in some manner decreasing, the action of the posterior head-inhibiting factor (Child, 1916; Buchanan, 1922; Hinrichs, 1924; Rulon, 1936, 1937; Miller, 1937). Evidence has also been presented which indicates that head frequency may be increased through the stimulation of the head-forming cells. Various agents, such as metabolic end-products, ethyl alcohol, dilute cyanide (Child, 1911*a*, 1912, 1916), anesthetics (Buchanan, 1922), and caffeine (Hinrichs, 1924), may decrease the head frequency of pieces from anterior levels and long pieces with certain exposure periods and concentrations. This inhibition is apparently due to a direct inhibitory effect of the agent on the head-forming cells, the different types of inhibited heads indicating that the most active regions are the most retarded in development.

Child and others have also found that, while cyanide and other agents may lower the head frequency of short anterior pieces, it may, at the same time, increase the head frequency of more posterior pieces. This increase is apparently due to partial elimination or blocking of the posterior inhibiting factor, while the head-forming cells, because of their normal lower activity, are less susceptible than the head-forming cells of more anterior regions.

In our experiments it has been found that different stocks, apparently because of a difference in physiological condition, respond somewhat differently to cyanide treatment. Animals which show a relatively high head frequency in the controls (Fig. 2) show a lowering in head frequency in pieces from *B*, *C*, and *D* levels. This decrease is particularly marked in *C* and *D* pieces. On the other hand, *A* pieces are apparently not affected by the treatment. It must be remembered, however, that the pieces were in the

agent for only 72 hours after section and that at the end of this time they were returned to well water. (If they had been kept for the entire period in the cyanide solution, very little, if any, new tissue would have developed.) Apparently, after the 72 hours' initial exposure the *A* pieces, presumably because of their initial higher activity, were able to recover, while more posterior pieces were unable to do so. These data then appear to support Child's hypothesis of differential recovery. At the same time, these data show the direct inhibitory effect of cyanide on the head-forming cells of pieces from the posterior levels.

Stocks which show a low head frequency under control conditions behave somewhat differently when pieces from the different levels are exposed to cyanide for 72 hours (Fig. 3). Pieces from *A*, *B*, and *C* levels respond by showing a slight but definite decrease in head frequency. Pieces from level *D*, however, show an increase in head frequency. Apparently, in these stocks *A* pieces, because of a different physiological condition, are unable to recover fully from the inhibitory effects of the agent. In *D* pieces the head frequency is apparently increased by a blocking or partial elimination of the posterior head-inhibiting factor, while the head-forming cells, because of a low activity at this level, are not inhibited as strongly by the direct action of the agent. From a consideration of all data it would then appear that the chief effect of cyanide is the inhibition of growth in the head-forming region, although other factors may enter in which tend to obscure the situation.

In considering the effects of methylene blue on head frequency in short transverse pieces of *Euplanaria*, it was found that in every experiment methylene blue brought about an increase in head frequency in pieces from *B*, *C*, and *D* levels. It was also found that stocks with a low head frequency in the controls were affected to a greater extent by this agent than were stocks with a high head frequency. At first it was thought that methylene blue was acting on planarian tissue in a manner directly opposite to that of cyanide. In other words, cyanide lowered the activity of the head-forming cells while methylene blue increased this activity. This assumption may be true in part, for no experiments have as yet been performed testing the respiratory metabolism of the pieces exposed to these two different agents. From the reconstitutional standpoint, however, it appeared that methylene blue, in the concentrations used, acted more as an inhibitory agent. Pieces treated with 1/200,000–1/300,000 methylene blue did not appear to have been stimulated as regards reconstitution. This was particularly true of pieces from the more anterior levels, where the amount of new tissue and the size of the eyespots appeared to be less than in the controls. Likewise, the pigmentation often appeared dark and diffuse, a condition commonly found in slightly inhibited worms. Another phenomenon resulting from methylene-blue treatment was that the reconstituted animals often appeared swollen and presented a blue, granulated appearance in the region of the gut.

It must also be remembered that 1/200,000–1/300,000 methylene blue will kill the pieces if they are allowed to remain in such a concentration much longer than 3 days. It has been pointed out that intact animals can survive from 5 to 7 days in 1/200,000 methylene blue but become heavily stained, inactive, and strongly insensitive before that time. Pieces become heavily stained, inactive, and insensitive by 3 days' exposure—phenomena which appear to result from an inhibitory, rather than a stimulating, action. Because of these and the foregoing observations it seems not unlikely that the increase in head frequency of pieces submitted to methylene blue for 72 hours may be due to an

elimination or blocking of the factors inhibiting head formation which have their origin at the posterior cut surface.

With methylene blue increasing head frequency and with cyanide, in general, lowering head frequency, it might be expected that the two different agents acting together would bring about a head frequency somewhere between that which would appear when the two were used separately. In other words, these two agents might mutually antagonize each other in their effects on reconstitution. To some extent this prediction has been borne out, but it certainly does not hold in many cases. In stocks which show a low head frequency in the controls (Fig. 6), the combined solution determines a head frequency of pieces from *B*, *C*, and *D* levels which is above that when cyanide alone is used and below that of methylene blue (Fig. 6, curve *O*). In *A* pieces, however, the combined solution determines a head frequency that is definitely lower than with cyanide alone. In animals which show a high head frequency in the controls (Fig. 7) the combined solution produces a head frequency of *B*, *C*, and *D* pieces which is slightly below that of cyanide alone, while *A* pieces are apparently unaffected.

The interpretation of these data is not easy, and the following suggestions may not be entirely correct. If we assume, however, that the chief effect of cyanide is by direct action on the head-forming cells and the chief effect of methylene blue is in blocking or eliminating the posterior head-inhibiting factor, then we can offer at least a partial explanation. We have seen that, in all cases where methylene blue alone is used, the head frequency is increased at all levels with the exception of *A*, which already is producing the maximum. When cyanide is used with methylene blue, in all cases (with the exception of *A* pieces in animals with a high head frequency) the head frequency is considerably lower than with methylene blue alone. It has been noted that the cyanide–methylene-blue solution stains the pieces just as intensely (or apparently as intensely) as methylene blue alone. This being the case, there seems to be no reason why the methylene-blue complement of the cyanide–methylene-blue solution should not block or eliminate the posterior head-inhibiting factor. With the head-inhibiting factor blocked, the head-forming cells would be released and would tend to grow if it were not for the cyanide. With the cyanide present in the solution, however, the head-forming region, although released from the posterior inhibiting factor, is inhibited directly by the cyanide. Worms which show a low head frequency are in a different physiological state from those with a high head frequency. Apparently, when the head-forming regions of pieces with a high-control head frequency are partially released from the effect of posterior cut surface, the reaction to cyanide is more intense than with pieces which show a low head frequency, as might be expected if increased cellular activity results. Only with this assumption can we explain why *B*, *C*, and *D* pieces of stocks which show a high head frequency show a greater decrease in head frequency with the cyanide–methylene-blue solution than with the cyanide alone. As regards *A* pieces, it has been found that in stocks with a high head frequency these levels are not affected by either cyanide or cyanide–methylene-blue solutions (Fig. 7). In this case it may be suggested that, because of a higher activity of the level, *A* pieces are able to recover from the 72 hours' initial exposure. In stocks with a low-control head frequency, *A* pieces are slightly inhibited by cyanide; and with the cyanide–methylene-blue solution they are even more inhibited. Apparently, in this case a release of any posterior effect plus the direct effect of cyanide gives such an intense reaction that complete recovery does not occur.

The experiments in which the intact animals were submitted to cyanide for 6 days

before sectioning and then allowed to reconstitute in methylene blue for 72 hours after section are of interest in that methylene blue restores, in part, the normal (control) head frequency in the cyanide-treated animals. Whether methylene blue in this case causes a direct increase in metabolism of cyanide-inhibited animals, a stimulation antagonizing the toxic effect of the cyanide, or an increase in head frequency by block or elimination of the posterior factor, can only be determined by further experiment.

SUMMARY

1. Pieces (one-eighth the length of the intact animal) from *B*, *C*, and *D* levels of 14–16-mm. worms, which under control conditions give a high head frequency, give a lowered head frequency when exposed to M/100,000 KCN for 72 hours after section.

2. In animals with a low-control head frequency in pieces from the posterior levels of the anterior zooid, exposure for 72 hours to M/100,000 KCN results in a lowered head frequency of pieces from *A*, *B*, and *C* levels but an increased head frequency of pieces f om *D* level.

3. Methylene blue, in concentrations of 1/1,000,000 for the full reconstitutional period to 1/200,000 for 60 hours after section, caused an increase in head frequency of *B*, *C*, and *D* pieces in all experiments.

4. In animals which show a low head frequency in the controls, an exposure of 72 hours to a combined solution of 1/300,000 methylene blue plus M/100,000 KCN determines a head frequency of pieces from *B*, *C*, and *D* levels above that for cyanide and below that for methylene blue alone. This combination also lowers the head frequency of *A* pieces to a greater extent than does cyanide alone.

5. In animals which show a high head frequency in the controls the combined cyanide–methylene-blue solution determines a head frequency in pieces from *B*, *C*, and *D* levels which is below that for cyanide alone, while *A* pieces are apparently unaffected.

6. Animals (with a high-control head frequency) treated with M/100,000 KCN for 6 days, sectioned, and allowed to reconstitute in well water show a marked decrease in head frequency in pieces from *B*, *C*, and *D* levels. Similar animals, sectioned and allowed to reconstitute in 1/200,000 methylene blue for 72 hours, show an increase in head frequency over those allowed to reconstitute in well water.

7. The evidence indicates that the action of cyanide on reconstitution in *Euplanaria* is chiefly through an inhibition of the head-forming cells, while methylene blue probably acts chiefly by blocking or eliminating head-inhibiting factors which arise at the posterior cut surface.

LITERATURE CITED

BODINE, J. H., and BOELL, E. J. 1936. Effect of methylene blue on respiration of blocked and developing embryonic cells. Proc. Soc. Exper. Biol. and Med., **34**:629.

———. 1937. The action of certain stimulating and inhibiting substances on the respiration of active and blocked eggs and isolated embryos. Physiol. Zoöl., **10**:245.

BROOKS, M. M. 1932. The effect of methylene blue on HCN and CO poisoning. Amer. Jour. Physiol., **102**:145.

———. 1933. The effect of respiratory poisons and methylene blue on cleavage of certain eggs. Collecting Net, **8**:95.

BUCHANAN, J. W. 1922. The control of head formation in *Planaria* by means of anesthetics. Jour. Exper. Zoöl., **36**:1.

CHILD, C. M. 1911*a*. Experimental control of morphogenesis in *Planaria*. Biol. Bull., **20**:309.
———. 1911*b*. Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. I. Jour. Exper. Zoöl., **10**:265.
———. 1911*c*. Studies, etc. II. *Ibid.*, **11**:187.
———. 1912. Studies, etc. IV. *Ibid.*, **13**:103.
———. 1914*a*. Studies, etc. VII. *Ibid.*, **16**:413.
———. 1914*b*. Studies, etc. VIII. *Ibid.*, **17**:61.
———. 1916. Studies, etc. IX. *Ibid.*, **21**:101.
———. 1920. Studies, etc. X. *Ibid.*, **30**:403.
———. 1924. Physiological foundations of behavior. New York: Henry Holt & Co.
CHILD, C. M., and WATANABE, Y. 1935. The head frequency gradient in *Euplanaria dorotocephala*. Physiol. Zoöl., **8**:1.
HINRICHS, M. A. 1924. A study on the physiological effects of caffein upon *Planaria dorotocephala*. Jour. Exper. Zoöl., **40**:271.
HUG, H. 1932. La intoxicacion por el acido cianhedrico. II. Accion antidotica del azul de metileno, sulfuro de sodio y nitrito de sodio. Rev. Soc. Argentina Biol., **8**:270.
MILLER, F. S. 1937. Some effects of strychnine on reconstitution in *Euplanaria dorotocephala*. Physiol. Zoöl., **10**:276.
RULON, O. 1936. The effects of carbon dioxide, the hydrogen ion, calcium, and experimental conditioning on reconstitution in *Euplanaria dorotocephala*. Physiol. Zoöl., **9**:170.
———. 1937. The effects of certain organic acids on reconstitution in *Euplanaria dorotocephala*. *Ibid.*, **10**:180.
WATANABE, Y. 1935*a*. Rate of head development as indicated by time of appearance of eyes in the reconstitution of *Euplanaria dorotocephala*. Physiol. Zoöl., **8**:41.
———. 1935*b*. Head frequency in *Euplanaria maculata* in relation to nervous system. *Ibid.*, p. 374.

STUDIES ON HETEROPLASTIC TRANSPLANTATION IN TRICLADS
I. CEPHALIC GRAFTS BETWEEN EUPLANARIA DOROTOCEPHALA AND E. TIGRINA[1]

(Ninety-eight figures)

JAMES A. MILLER
University of Chicago and Marine Biological Laboratory

IT IS commonly recognized by experimenters that by the production of abnormal relationships between parts it is often possible to isolate some of the factors which operate in the development and maintenance of the normal characteristics of organisms. The large volume of work on extirpation, explantation, and transplantation is evidence of the possibilities of this type of experimental procedure.

In the study reported here, interspecific transplantation among triclad flatworms was selected as a method of studying certain phases of "dominance," a phenomenon which has been recognized in the zoölogy laboratory of the University of Chicago for the last quarter of a century (Child, 1911*a*) and has recently come to the attention of embryologists in various parts of the world (Huxley and de Beer, 1934; Huxley, 1935; Weiss, 1935; Strelin and Trifonova, 1935). For a brief review of the characteristics of the dominant region of the simpler invertebrates see J. A. Miller (1937).

Most of the earlier workers on transplantation in the lower invertebrates have been concerned with problems other than those attacked here. However, numerous instances of dominance may be gleaned from their data. Among coelenterates, Browne (1909) found that small pieces of the hypostome region of *Hydra* will determine a new apical region in the host and modify its polarity. Rand, Bovard, and Minnich (1926) confirmed these results and demonstrated the "inhibitory dominance" of such a graft. Mutz (1930), using *Pelmatohydra* and *Hydra*, showed that the effects are not species specific. Burt (1934) found a gradient in the ability of grafted rings of tissue from different levels of *Pelmatohydra oligactis* to dominate the host. Child (1929*b*, 1932) studied dominance in *Corymorpha* by means of grafting. In this form pieces from all levels may induce a new hydranth and stalk, but distal pieces are most potent. It is not even necessary to implant any tissue, for laceration of the edges of an incision is sufficient stimulus to cause the cells in the vicinity to produce a hydranth (Child, 1927, 1929*b*).

Among the flatworms, Moretti (1911), using *Planaria torva*, found that only grafts of the head could persist and maintain the autonomic movements. He figured and described cases of inhibition of regeneration after removal of the host head and of induction of a pharynx by grafts of the cephalic region but did not discuss them. Rand and Browne (1926) reported four cases in *Euplanaria tigrina* (syn. *Planaria maculata* [Kenk, 1935]) in which there was no regeneration of the host head after its removal close to a cephalic graft. Gebhardt (1926) reported a few cases in *Planaria lugubris* in which there were outgrowths of the body as a result of grafting. Goetsch (1926, 1929) found that a graft

[1] The writer wishes to express very great appreciation for the interest and inspiration of Professor C. M. Child in this work, as well as for proposing this line of investigation and for helpful suggestions during its progress.

of a regenerating head could prevent the development of a host head and eventually take its place. When a small portion of the host was isolated with the graft, it not only prevented development of head and tail but seemed to grow at the expense of the host. Santos (1929, 1931) has made the most complete study of dominance by the grafting technique. He found that ganglionic grafts of *Euplanaria dorotocephala* in prepharyngeal regions could induce the development of postcephalic structures. In pharyngeal regions they might induce a pharynx, and in postpharyngeal regions two pharynges might be induced with the one anterior to the graft in reversed polarity. The indications were that the ability of the graft to inhibit the regeneration of a host head depends upon the level of implantation. These effects were not species-specific, for heteroplastic transplants between *E. dorotocephala* and *E. tigrina* gave the same results. Grafts other than from the head did not possess this reorganizing power.

Steinmann (1933), using *Dendrocoelum lacteum*, reported a case in which a ganglionic graft placed in the tail developed two small pharynges and more or less complete sets of reproductive organs. Finally, Okada and Sugino (1934) reported cases of dominance of host by graft in the Japanese *Planaria gonocephala*, in which not only pieces containing the cephalic ganglia but also those from between the head and pharynx could induce pharynges in postpharyngeal regions of the host.

In the experiments reported here heteroplastic transplants were used as a means of distinguishing between implant and host in order to secure more exact data on the regeneration of the graft, the ability of grafts from various levels of the donor to maintain themselves, the differences in inductive capacity of grafts from various levels, and the differences in resistance of various host levels to the domination of grafts.

MATERIAL

The animals used in these experiments were *E. dorotocephala*, collected in spring-fed streams near Rockford and Cary, Illinois, and Valparaiso, Indiana; *E. tigrina* (Kenk, 1935; syn. *P. maculata*) from Valparaiso; and *E. tigrina novangliae*[2] from a pond in Falmouth, Massachusetts. These three forms are closely related anatomically and proved well adapted to the requirements of the experiments reported here. Takes were readily secured; yet there were sufficient external differences to distinguish between graft and host tissues, a fact which permitted accurate data on the fate of the graft and on its inductive capacity. Most of the transplantations were between *E. dorotocephala* and *E. tigrina novangliae*.

The external differences by which these three forms may be distinguished are listed below. They consist of differences in size, shape, pigmentation, and appearance of the digestive tract and of the photoreceptors. *Euplanaria dorotocephala* (Pl. I, Fig. 1) is the longest of the three; *E. tigrina* (Pl. I, Fig. 2), the shortest; and *E. tigrina novangliae* (Pl. I, Fig. 3), the widest. There are consistent differences in shape of head. The head of *E. dorotocephala* is pointed with long "auricles" and a slight constriction in the "neck region"; that of *E. tigrina* is blunt with short and very wide "auricles" and with very little constriction in the "neck region."

With respect to pigmentation the three forms are quite distinct. The pigment of *E. dorotocephala*, almost black in nature and dark to light brown when kept in the labora-

[2] *Euplanaria tigrina* and *E. tigrina novangliae* are so closely related that Kenk (1935, p. 85) stated that "the copulatory organs afforded no means of discriminating between them" but that on the basis of the external differences they may possibly be subspecies.

tory, is distributed over the surface of the animals in small, dark granules against a brown or tan background. The ventral surface is a grayish hue because of the lighter background than that on the dorsal surface. The dorsal surface of *E. tigrina* (as represented at Valparaiso, Indiana) is finely maculated with yellowish-orange pigmented areas against a tan background. The ventral surface of this form is white with gray pigment areas, giving a light-gray effect. The coloration of *E. tigrina novangliae* is very variable; therefore, only one type was used in these experiments. The dorsal surface of this type is characterized by large pigmented areas, some of which are light and some of which are dark brown, interspersed with occasional colorless areas, and by a mid-dorsal light stripe, along each side of which there is an irregular row of darker spots. The ventral surface of these planarians is much darker than that of *E. tigrina maculata* and has a brownish tinge.

The photoreceptors of the three can be distinguished by the differences in the size and shape of the unpigmented areas. Those of *E. dorotocephala* are elongated and set in such a fashion that the anterior parts are closer together than the posterior. Those of *E tigrina* are very large and are placed with their axes parallel to each other. The crescentic pigmented areas of the photoreceptors are completely visible in this form. In the case of *E. tigrina novangliae* the unpigmented areas are less perfectly oval, are smaller, and do not completely expose the pigmented areas.

The branches of the digestive tract of living planarians may be readily observed by allowing the animals to feed on clotted or dried blood. Those of *E. dorotocephala* are characterized by extensive anastomoses and by the formation of accessory longitudinal trunks in connection with both anterior and posterior rami of the intestine (Hyman, 1925). On the other hand, the branches of the intestine of *E. tigrina* are more regular and do not develop the anastomoses and accessory trunks such as are characteristic of the other species.

Preliminary transplants were made according to the method described by Santos (1931); however, it was found that the degree of anesthesia could be more easily controlled and that recovery was more rapid when a physical agent (low temperature) was used instead of a chemical one (chloretone). Likewise, it became apparent that the use of rectangular grafts containing the same volume of tissue as the triangular ones used by Santos resulted in a higher percentage of "takes," since incisions of this shape had less tendency to cause the host to tear apart at the angles. With these modifications, very high percentages of unions were obtained, usually between 75 and 100 per cent.

PROCEDURE

Except in cases in which grafts were made in the cephalic region the prospective hosts were decapitated from 2 to 4 hours before operation in order to reduce their activity. The recipient was placed, together with a small amount of well water, on a clean, grease-free glass square resting on a pan of ice from a refrigerator. When the host was sufficiently anesthetized, a rectangular window was removed with the aid of two very sharp half-spear dissecting needles under a low-power (55-mm. objective) binocular microscope. Because of the strong contraction of the cut surfaces, it was found necessary to make the graft considerably smaller than the window into which it was to be placed. It was placed in the desired orientation, and the excess water drained off the plate. Then a Petrie dish with vaselined edges was inverted over the plate, making an airtight chamber. This was placed in the dark for 8–12 hours, after which the "takes" were transferred

to finger bowls with approximately 200 cc. of well water, and were measured and recorded. At this time controls from the same stock were cut with windows as nearly like those of experimentals as possible, but no graft was inserted. Both experimental and control animals were fed beef liver or dried blood, and the water was changed twice a week. Subsequent observations were made at weekly intervals. Certain of the specimens were killed in a 0.5 per cent solution of HNO_3 and fixed in a saturated solution of $HgCl_2$ in a 0.6 per cent solution of NaCl (Willier, Hyman, and Rifenburgh, 1925). A report of the histological study of these grafts will be made at a future date.

Large specimens of the three forms were used. Those of *E. dorotocephala* were between 17 and 22 mm. in length; those of *E. tigrina* between 11 and 14 mm.; and those of *E. tigrina novangliae* between 11 and 16 mm. The grafts usually ranged in size from 0.8 × 1.0 mm. to 1.1 × 1.3 mm. The few that were smaller occasionally were resorbed, while in larger ones the frequency of takes was low.

Three regions of the body were selected for study: the head region, which contains a concentration of nervous elements; the pharynx, which contains highly differentiated muscular and nervous elements; and the tail, which is the actively growing but least differentiated portion of the animal. Grafts were taken from each of these regions of the donor and transplanted into each of three regions of the hosts. All possible combinations of dorsal and ventral with anterior and posterior surfaces of graft and host were made.

In order to make the results comparable, grafts were cut from, and implanted into, as nearly the same levels as possible. All cephalic grafts and windows in the hosts were cut with the anterior surface just in front of the unpigmented areas of the eyes; pharyngeal grafts and windows were cut to include the muscles attaching the proximal end of the pharynx to the body wall. Tail grafts and windows were cut from the most posterior tips of the tail. They were made slightly wider at the anterior end to include as much of the growing region as possible. In addition, 20 cephalic grafts were implanted just behind the eyes for comparative purposes. In this report only the results of grafts from the cephalic region will be discussed.

Since neither the head nor tail levels of this study corresponded exactly to the regions used by Santos (1929, 1931), a different terminology has been employed in referring to them. The head grafts in this study did not contain the entire ganglionic region and therefore are called "cephalic," rather than "ganglionic," grafts. Similarly, the most posterior level used is designated as the "tail" region, and grafts from it as "tail grafts," because they were located more posteriorly than the postpharyngeal level of Santos. The "tail" represents the more posterior levels of the posterior zooid region. The terminology used in describing the orientation of the grafts is a modification of that introduced by Harrison (1921) and widely used in amphibian experiments. When the dorsoventral axes of graft and host coincided, the implant is referred to as a "dorsodorsal"; when reversed, it is called a "dorsoventral." When the anteroposterior axes coincided, the graft is an "anteroanterior"; when reversed, an "anteroposterior."

Records were kept on mimeographed sheets with columns for date, measurements (graft length and width; host length and width), drawings, and notes. The notes consisted of observations on the extent of union between graft and host, the development of the transplant, the behavior of host and graft, and feeding responses. In the more recent experiments the weekly drawings were supplemented by photographs taken with a Leica camera and a 1:1 copying attachment. In most cases the outlines of the digestive tract were recorded after feeding the specimens dried blood.

EXPERIMENTAL

GENERAL OBSERVATIONS ON GRAFTS AND CONTROLS

A total of 412 cases of successful transplantation of cephalic pieces were studied. Of these, 105 were placed in the head region, 20 in the anterior prepharyngeal region, 113 in the pharyngeal region, and 174 in the tail. No species differences in inductive potency of grafts or in resistance to induction by the host, as indicated by Santos (1931), were noted. For this reason, and in order to avoid excessive length in the experimental section, no distinctions between species will be made in the descriptions.

Takes.—Although in all types of transplants reported here 100 per cent takes were obtained upon occasions, in general union occurred more commonly with *E. tigrina* as host than with *E. dorotocephala*. Of the three host regions employed, the tail gave the lowest percentage of takes and the head the highest. Of the four cut surfaces of the graft, the anterior united with the host the least frequently and the posterior the most.

Behavior.—Implants in the head in dorsodorsal anteroanterior orientation which united by all surfaces often became immediately incorporated into the host, and normal behavior of the host became re-established within 12 hours after operation. When possessing a free surface or placed in other orientations, grafts in the head usually maintained their own individuality but did not extend their dominance appreciably into the surrounding tissue. On the other hand, when placed in the pharynx region, cephalic grafts controlled the movements of more posterior portions of the host but were unable to gain ascendancy over the more anterior regions. The orientation and degree of union did not greatly affect the ability of grafts to control host movements when placed in the pharynx or tail. Cephalic grafts placed in the tail region controlled the movements of the host both anterior and posterior to the level of implantation.

When two or more heads were present in the same animal, as was the case in most of the experimental animals, considerable difficulty was experienced in inducing the worms to feed. The stimulation by food was considerably greater in these than in normal worms; and, though they would crawl over the food again and again, the pharynx was unable to start feeding before being dragged off by the very active heads. In some cases it was possible to induce these animals to feed by covering the containers with a black cloth. In the absence of stimulation of the photoreceptors the activity of the heads was sufficiently reduced to permit the institution of feeding-responses by the pharynx.

Regeneration.—In all levels a free surface was necessary for regeneration of the normal head shape by the implant. The free surface likewise determined its polarity, with modifications up to 180° not uncommon.

Union of the digestive tracts.—Union of the intestinal diverticula of the graft with those of the host was necessary for the persistence, as well as for the growth, of the graft. However, even in starving grafts, regeneration of lost parts from a free surface occurred, and the ability to induce host structures was not appreciably reduced.

Disintegration of host tissues in the neighborhood of implants.—In certain grafts in the head, prepharyngeal, and pharyngeal regions disintegration of tissue anterior to the level of implantation occurred. In most of these cases no food was observed, after feedings, in the regions where disintegration occurred later. In the rest, the digestive tracts in the affected regions reunited with small side branches and often received little or no food. In all, the typical changes which have been observed in starving planarians (including disintegration of the head) were noted. Accordingly, it is suggested that the disintegration of the heads in these cases might have been caused by starvation.

In many of the cases in which union of graft and host was only partial, sooner or later the host tissue on one side of the graft disintegrated and the implant which had projected dorsally from the host came to lie in the same plane as the host. Disintegration of this localized area is thought to have occurred as a result of the activities of the graft, which, in applying itself to the substrate, constricted the host tissue lateral to it. Whether disintegration was due to mere mechanical pressure or to some secondary effect of the constriction, such as interference with food supply and waste-removal, cannot be said.

Controls.—In the controls, in which a window was made but no graft was inserted, the cut surfaces united within a few hours after the operation. In the head the anterior and posterior surfaces usually united, while in the pharyngeal and tail regions it was the lateral surfaces of the windows which fused. Usually within 2 weeks new tissue had filled in the injury completely, and the structures which had been removed were replaced. There were very few exceptions to this procedure; they will be treated in their appropriate places in the sections which follow.

GRAFTS IN THE HEAD REGION (105 CASES)

Dorsodorsal anteroanterior orientation of graft (24 cases).—The behavior of cephalic grafts implanted in the head region depended principally upon their orientation and the degree of union with the host. When both dorsoventral and anteroposterior axes coincided, grafts and hosts united along all surfaces (22 of 24 cases). Within 12 hours after operation the experimental animals were observed to crawl normally and react normally to food; whereas in the case of the controls, normal locomotion and feeding responses did not appear until regeneration had proceeded as far as the development of approximately the normal head shape and photoreceptors, a week or more later. In almost all cases the photoreceptors of the implants happened to lie in the eye-level of the host. In these, proliferation of host tissue was at a minimum and no host eyes[3] were reconstituted (Figs. 4, 72). In contrast to the experimentals the control planarians in every case regenerated the tissue removed, including the two photoreceptors. In 3 cases supernumerary eyes were developed (Figs. 5, 6).

When the photoreceptors of the implants did not happen to lie in the eye-level of the host, one or more eyes were reconstituted by the host (Fig. 7).

In 4 cases the tip of the host head disintegrated, leaving the anterior portion of the graft exposed. These resulted in a chimaera-type head of which the mesial part was of one species and the "auricles" and sides were of the other (Fig. 8).

In all grafts which were kept 3 months or more, there was a gradual replacement of graft epidermis and of graft photoreceptors by those of the host. Whether other tissues were involved is not known. This replacement, which was observed not only in grafts in the head region but also in those in the other two levels used in this study, proceeded as follows. Usually within 1 week after implantation the edges of the transplant became less clearly defined and a ring of unpigmented tissue was observed surrounding the graft. This ring of new tissue (regenerating tissue in these planarians does not become heavily pigmented for 2 weeks or more) was observed gradually to encroach upon the graft during the next 6–8 weeks until all that could be seen of the implant was a few darker pigment spots between the photoreceptors. Eventually they, too, disappeared. Meanwhile, pigment spots characteristic of the host species developed around the

[3] Strictly speaking, planarians possess photoreceptors and not eyes, since they have no apparatus for focusing an image upon the photosensitive cells. However, in this report "eye" is used in its less exact sense as a synonym of photoreceptor.

periphery of the new tissue, showing that the cells replacing those of the graft were of host and not of graft origin. It was not possible to follow the process of replacement of the photoreceptors with the same degree of assurance as in the case of the epidermis. However, the change in shape of the unpigmented areas from that characteristic of the graft to that of the host seemed to occur following the depigmentation of the surrounding tissue. The black crescentic pigment areas of the photoreceptors also changed shape gradually but by such imperceptible stages that in most cases the process could not be followed. In 3 cases the black pigment areas of the photoreceptors of the graft were observed to decrease in size gradually, and later a new pigment spot appeared in the unpigmented area of each eye. This increased in size until it fused with that of the graft. Whether this represents the usual situation is not known.

GRAFTS IN THE HEAD REGION

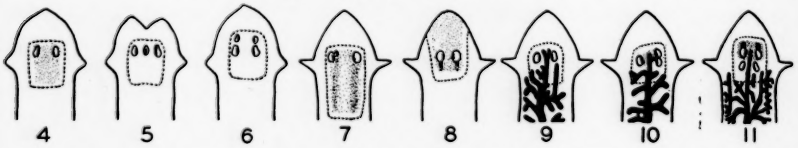


FIG. 4.—*E. dorotocephala* into *E. tigrina novangliae*. DDAA.* Union complete. 31 days. Epithelia of implant were eventually replaced by host tissue.

FIGS. 5 AND 6.—*E. tigrina novangliae*. Controls 31 days. FIG. 5.—Three photoreceptors have developed in place of the two which were removed. FIG. 6.—Four photoreceptors have developed in the new tissue.

FIGS. 7 AND 8.—*E. tigrina novangliae* into *E. dorotocephala*. DDAA. 35 days. FIG. 7.—Union complete. Large graft (1.4 × 0.8 mm.). Eye appeared in new tissue left of implant 2 weeks after grafting. Left graft eye became reduced in size and subsequently fused with it. FIG. 8.—Anterior edge of implant failed to unite with host. Host head disintegrated anterior to implant, resulting in chimaera-type head.

FIGS. 9, 10, AND 11.—*E. tigrina novangliae* into *E. dorotocephala*. DDAP. Union complete. FIG. 9.—35 days. A small photoreceptor appeared anterior to right graft eye 1 week after implantation and disappeared 2 weeks later. Graft epithelia completely replaced by new tissue. Note that digestive tract extends farther anteriorly than normally. FIG. 10.—14 days. One eye appeared posterior to left graft eye 1 week after implantation. Four weeks later new tissue had completely replaced graft epithelia. FIG. 11.—28 days after operation. Two eyes have developed in new tissue posterior to implant.

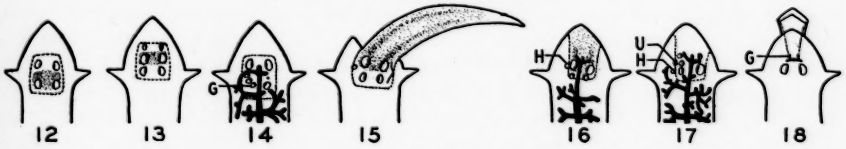
Dorsodorsal anteroposterior orientation of graft (64 cases).—Cephalic transplants placed in the head region with their anteroposterior axes reversed were some of the most interesting of all the types studied. The percentage of cases of complete union (42 per cent) was less in this class of grafts than in those in which the anteroposterior axes coincided. When reversed, the relatively thick posterior surface of the graft was in contact with the thin anterior surface of the host window, and vice versa. It is thought that this was chiefly responsible for the proportionally fewer cases of complete union in these grafts than in the class just reported.

In cases of complete union the grafts showed little independent activity, and the proliferation of tissue resulting from the implantation was so slight that in 5 cases no outgrowth was noted and in the rest it was very small. In all but 4 cases more than one

* In these and subsequent figures the following conventions are used: graft tissue stippled where distinguishable, approximate extent of new tissue indicated by broken lines, invaginated portions by alternating dots and dashes, main intestinal branches by solid black. Time after operation given in days. Orientation of implants: DDAA = dorsodorsal, anteroanterior; DDAP = dorsodorsal, anteroposterior; DVAA = dorsoventral, anteroanterior; DVAP = dorsoventral, anteroposterior.

photoreceptor developed. Of the exceptional cases, 1 developed a very small eye which later regressed (Fig. 9), and the other 3 cases regulated a permanent photoreceptor (Fig. 10). The usual result was the development of one or more photoreceptors in the new host tissue either anterior or posterior to each eye of the graft. In 3 cases one supernumerary eye developed anterior to each eye of the graft, and in 3 two eyes developed posterior to those of the graft (Figs. 11 and 12). There were 3 cases in which four eyes developed, two of them anterior and two posterior to those of the implant (Fig. 13). In other cases the supernumerary photoreceptors were not as regularly arranged, although they never appeared very far from the region of the mesiolateral axis at which eyes are found normally (Fig. 14). It was noted that the supernumerary eyes usually appeared considerably later than did the regenerating eyes of the controls, with a delay of 2 or 3 weeks not uncommon.

GRAFTS IN THE HEAD REGION



FIGS. 12, 14, 15, 16, AND 17.—*E. tigrina* into *E. dorotocephala*. FIGS. 13 AND 18.—*E. tigrina novangliae* into *E. dorotocephala*. In all but Figs. 15 and 18 union was complete. DDAP.

FIG. 12.—35 days. Two eyes have developed in new tissue posterior to implant.

FIG. 13.—28 days. Two eyes have developed in new tissue anterior to implant and two more posterior.

FIG. 14.—56 days. Two eyes appeared anterior to implant 1 week after implantation. Three more developed later in new tissue between graft and host eyes. Left graft eye (G) has become reduced in size.

FIG. 15.—Host disintegrated anterior to implant. 42 days. Tail-like outgrowth has regenerated from free graft surface. Two eyes developed in new tissue opposite graft eyes 1 week after implantation. Unpigmented area on left and projection on right developed later.

FIGS. 16 AND 17.—7 and 49 days, respectively. FIG. 16.—Unpigmented portion of left host eye (*H*) not completely removed. Two eyes have appeared posterior to graft. FIG. 17.—Graft epithelia completely replaced by new tissue. Graft eyes have fused with two supernumerary eyes shown in Fig. 16. Five additional eyes developed anterior to graft eyes but have been reduced by fusions to three. Unpigmented projection (*U*) had developed to left of implant.

FIG. 18.—35 days. Posterior surface of graft failed to unite with host. Resulting tubular outgrowth lined with ventral epithelium, projects from ventral surface of host. Pigment areas of graft eyes (*G*) visible. Two eyes present in new tissue posterior to implant.

A comparison of the conditions of the digestive tracts of the experimental and control animals showed that in the former, as a result of reversing the anteroposterior axis of the graft, the intestine extended into the head of the compound well anterior to the photoreceptors of the implant. In the controls the most anterior branch ended between the regenerated eyes. Usually when there were side branches of the digestive tract anterior to the eyes of the graft, supernumerary eyes developed anterior to those of the implant. However, the correlation between diverticula and number of eyes was not sufficiently close to be interpreted as the determination of the latter by the former. It seems probable that farther anterior extension of the digestive tract in these specimens merely furnishes a greater supply of nourishment to the more anterior levels of the head and allows the development of eyes which might otherwise suffer from competition with the graft.

The presence of a free surface in cases of incomplete union resulted in greater activity on the part of the implant and considerably greater proliferation of tissue as compared

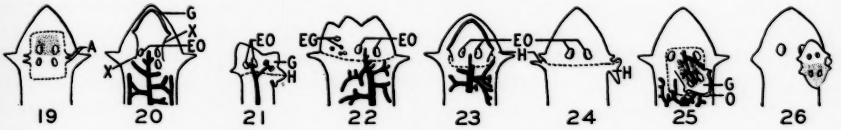
with cases of complete union. In 3 cases the host tissue anterior to the implant disintegrated within a day or two after grafting, leaving the posterior surface of the graft free. In two of these the implant retained its original polarity and showed almost continuous activity, contracting and extending entirely independently of the movement of the host. An outgrowth resembling a tail developed from the free surface of the implant, and two eyes formed in the regenerating host tissue directly behind and opposite those of the graft (Fig. 15). In the third case fusion of graft and host occurred without any appreciable proliferation of new tissue, as was the case in grafts in which both dorsoventral and anteroposterior axes coincided. However, within a week two supernumerary eyes had appeared posterior to those of the implant (Fig. 16). During the next 3 weeks five more developed anterior to them, and in addition there was by this time an unpigmented projection suggestive of an abortive cephalic lobe to the left of the graft. In the ensuing 4 weeks the eyes of the graft fused with the two supernumerary eyes that were posterior to them, and fusions were occurring in the eyes which had developed anterior to the graft (Fig. 17). Thus, even though the implant had its polarity reversed as a result of its position, nevertheless it produced a marked effect upon the host head.

In 19 cases a part of one surface of the graft failed to unite with the host. From this free surface and the cut surface of the host developed a tubular outgrowth around which later appeared supernumerary eyes. Usually it was possible to determine by the position of the developing eyes that the free surface was anterior, even though in some cases it developed from the lateral or posterior surface of the graft. In most cases the outgrowth extended from the upper surface of the host and was surrounded by dorsal epithelium with the ventral surface on the inside of the tube. In one case, however, it extended ventrally and the dorsal surface was inside (Fig. 18). Figure 19 shows a case in which there were two free surfaces—one a small portion of the left, and the other a smaller portion of the right side of the implant. The two free surfaces developed outgrowths resembling cephalic lobes with their unpigmented sensory areas, and a second pair of photoreceptors developed in the regenerating host tissue opposite the anterior surface of the graft.

In 8 cases the entire anterior surface of the implant remained free. In these the anteroposterior axes of the transplants were not affected. Each graft regenerated a normal or nearly normal head, and the host developed a second head from the free edge of the window directly opposite the graft. This grew rapidly until it reached the size of the head of the implant, and during all subsequent observations both remained the same size except when conditions were altered. The presence in the ganglionic region of the host of an *active* graft with its anteroposterior axis reversed was sufficient, in all cases except one, to inhibit completely the development of eyes anterior to it. It appears that grafts that were able to escape the domination of the host and retain their own individuality, because of the free anterior surface, so altered conditions in the head of the host that the physiological level at which eyes are produced did not develop. On the other hand, it should be noted that, even when an implant lacking a free surface became dominated by the host to the extent of having its polarity reversed, it still exerted a modifying influence on the host, extending the physiological level at which eyes could develop over a much wider area than is normally found in these species. In this connection it might be recalled that Abeloos (1930) reports finding specimens in his stocks of *P. gonocephala* with supernumerary eyes usually arranged, as was the case in these experiments, in pairs extending toward the anterior margin of the head. He listed other reports of

supernumerary eyes (Janichen, 1897; Lang, 1913) and attributed them to senescent conditions, since they usually appeared in the largest specimens. However, Child (1921) has shown that susceptibility to inhibiting agents acting during head regeneration, as indicated by modifications of head-form and position or absence of eyes, decreases from the median region laterally. This indicates a higher gradient level in median than in lateral regions. Consequently, the hypothesis that the degenerative changes associated with senescence should cause an increase in the breadth of the area in which eyes are found seems improbable. In the present studies it was noted repeatedly that, when

GRAFTS IN THE HEAD REGION



FIGS. 19–22.—*E. tigrina* into *E. dorotocephala*. FIGS. 23–25.—*E. tigrina novangliae* into *E. dorotocephala*. FIG. 26.—*E. dorotocephala* into *E. tigrina novangliae*. In all but Figs. 19 and 26 anterior surface of graft failed to unite with host. Orientation of all DDAP.

FIG. 19.—21 days. From each lateral free surface two auricle-like structures (*A*) developed. Two eyes have appeared posterior to graft.

FIGS. 20 AND 21.—28 and 56 days, respectively. FIG. 20.—Tubular outgrowth, of which the graft forms the anterior half, has developed. Three eyes illustrated (*EO*) are in host portion of outgrowth. Ventral surface of implant (*G*) visible just behind anterior edge of outgrowth. Disintegration of anterior part of host to *X–X* and of region of union between two components of tubular outgrowth resulted in condition shown in Fig. 21. Right remnant of host head (*H*) and graft (*G*) form right component, and host portion of outgrowth forms the other. Unpigmented areas of right eyes have fused. Graft epithelia have been completely replaced by host tissue.

FIG. 22.—28 days. Tubular outgrowth with two eyes (*EO*) developed and, by disintegration of more anterior parts of host and region of union on left, produced a wide, flat composite head. Double unpigmented area appeared at line of junction between graft and host. Eye has appeared beside right graft eye. Graft epithelia completely replaced by new tissue.

FIGS. 23 AND 24.—7 and 28 days, respectively. FIG. 23.—Graft (*G*) almost entirely hidden behind tubular outgrowth of new tissue in which are two eyes (*EO*). Digestive tract did not reach host or graft head. FIG. 24.—Host and graft heads disintegrated (host by fourteenth day after implantation, graft by twenty-first day), leaving only host portion of outgrowth and auricles of original head (*H*).

FIG. 25.—14 days. Host portion of tubular outgrowth (*O*) lies behind graft (*G*). In contrast to Fig. 23 graft and host digestive tracts united. During 5 weeks following, before it was fixed, graft and host portions of outgrowth continued to increase in size.

FIG. 26.—Graft united to host by posterior part of ventral surface only. 14 days. Digestive tracts of graft and host failed to unite. Head with reversed polarity developed from posterior edge of graft. Graft decreased in size rapidly and separated from host 3 or 4 days after this drawing was made.

supernumerary eyes appeared, they developed in the new tissue during the period of active regeneration of the host, and that later there was a tendency for the eyes to regress and be resorbed.

In 3 cases the portion of the host anterior to the graft was devoid of intestinal branches. During 4 or 5 weeks these portions gradually decreased in size, lost their pigmentation, and finally disintegrated. The graft head and that of the host which had developed behind the graft now became the functional heads of the compound (Figs. 20 and 21). In 2 of the 3 cases the digestive tract of the graft did not unite with the host,

and in these cases the graft also regressed. In 1 case it became an appendage on the left side of the second host head (Fig. 22); while in the other it, too, disintegrated, leaving the second host head at the anterior end of the host (Figs. 23 and 24).

Figure 25 shows the result when both the graft and the anterior portion of the host re-established connections with diverticula from the host intestine. In this case the graft and the head behind it grew until attaining a size practically equivalent to that of the original host head. A postcephalic outgrowth of tissue similar to that described by Santos (1931) was induced by both the graft and the host head.

In 2 instances the amount of union between implant and host was very slight. In both, even though the grafts rapidly decreased in size and gave evidence of starvation from the first, they regenerated "auricles" and the triangular head shape which is normal for the species used. In 1 case (Fig. 26) the graft developed a second head on its free posterior surface, reminding one of the findings of Morgan (1903) and Child (1911*b*) that very short transverse sections of planarians sometimes develop bipolar forms.

Dorsoventral anteroanterior orientation of graft (*16 cases*).—When transplants were made in the head region with the dorsoventral axis reversed, they usually united with the host by all four cut surfaces. Within 12 hours after implantation the compounds were actively crawling. However, in contrast to the first described grafts, the anterior ends of these were continuously raised above the substrate as if the ventral longitudinal muscles of the graft were in a state of contraction.

In 5 cases the anterior tip of the host disintegrated within the first week after implantation. The presence of the graft prevented the regeneration of the host, and the implant itself reproduced the missing parts from its anterior surface (Fig. 27 and Pl. I, Fig. 28).

In all cases in which the dorsoventral axis was reversed, there was some proliferation of tissue between the graft and the host. In 4 cases both graft and host were involved, with the resulting development of one or two additional heads. The dorsal one was composed of the dorsal surface of the host and the ventral surface of the graft, and the ventral one, below the host head, consisted of the dorsal surface of the graft and ventral surface of the host. In 2 cases the ventral supernumerary head was bipolar.

In two cases the graft and host united by the dorsal surfaces only. In these the graft regenerated an anterior end, auricles, a functional pharynx, and a tail. During the 10 months that they were under observation the grafts, which showed greater muscular activity than their hosts, likewise showed relatively greater growth. By the end of the period, the two components were approximately the same size and crawled equally well whether the host ventral surface or that of the graft was in contact with the substrate (Pl. I, Fig. 29). After feeding them blood, it was possible to determine that the digestive tracts of graft and host were anastomosed, but behavior tests indicated that there were no extensive connections between the nervous systems of the components. A remarkable feature of these examples—one by which they differed from the others of this series—was that the grafts retained their specific characters during the entire period of observation. This condition will be treated in the discussion.

Dorsoventral anteroposterior orientation of graft (*11 cases*).—Only a few grafts were made with their dorsoventral and anteroposterior axes reversed. As was true in the other cases of inversion of the dorsoventral axis of the graft, there was a tendency for the anterior edge of the implant to fail to unite with the host. In only 3 instances were there complete takes. These resembled those of the series mentioned above in their behavior,

crawling with their heads continuously raised from the substrate. There was more or less proliferation of tissue between the graft and host, and in 1 case this was sufficient to inclose the graft completely. In this animal, eyes regenerated in the host and the graft regressed until it became a small projection on the anterior part of the host head. In the others the graft remained and no host eyes developed.

Incomplete takes usually failed to establish connections with the digestive tract of the host. Although they regenerated a more or less normal head, and in one instance a tail as well, they rapidly decreased in size and did not prevent the regeneration of a head by the host in cases in which it had previously disintegrated. In one incomplete take the digestive tract of the implant united with that of the host and the graft completely prevented any regeneration at the anterior end of the host (Pl. I, Fig. 30). In these, as well as in other dorsoventral grafts, there was no indication that the dorsal or ventral epithelia were modifiable.

Grafts in the anterior prepharyngeal region (20 cases).—Since the head contains the enlarged ganglionic part of the nervous system, there is the possibility that the resistance to induction at this level is a function of the amount of nervous tissue present. Accordingly, 20 grafts were implanted just behind the head. Not all possible combinations of dorsal and ventral with anterior and posterior surfaces were used, but the results were sufficient to show that there was no marked reduction in resistance in this level.

When grafts in normal orientation united completely with their hosts, there was no proliferation of tissue; and the only indication of induction was the appearance of a projection at each side of the implanted eyes (Pl. I, Fig. 31).

Figures 32 (Pl. I) and 33 show cases in which the grafts in normal orientation united by their posterior and a part of their lateral surfaces. A small postcephalic outgrowth developed in each case and subsequently tore through the right side of the host. The two differ in that in Figure 32 the graft and host heads are approximately equivalent, whereas in Figure 33 the host head has become reduced in size as a result of insufficient food reaching it from the branches of the digestive tract.

In Figure 34 we see a perfect take in which the anteroposterior axis was reversed. There was a slight proliferation of tissue around the graft, but otherwise no modification of the structure of the host was observed. Implanting the graft in reversed dorsoventral polarity usually resulted in union with the dorsal surface only. The grafts regenerated in nearly normal shape and induced a very small outgrowth of tissue (Figs. 35 and 36), producing a structure which resembled more or less several of Santos' (1931) figures (Figs. 4, 5, 6, 9, 13, and 14). There were two complete takes of dorsoventrally reversed grafts. In both of these a head developed composed of dorsal surface of the host and ventral surface of the graft (Fig. 37). In one the head developed from the anterior edge of the window and posterior edge of the graft, and in the other vice versa. In none of the grafts in this level was there any marked reduction of host resistance to reorganization. Accordingly, it may be concluded that the differential from anterior to posterior in resistance to induction is not dependent upon the presence or absence of certain structures concentrated in the most anterior levels, such as the cephalic ganglia.

GRAFTS IN THE PHARYNGEAL REGION (113 CASES)

When a cephalic graft was implanted into the pharyngeal region, the regenerating pharynx appeared some distance posterior to the original location, whereas that of the controls developed in the new tissue which marked its original position. Since the

pharynges of the experimental animals developed out of their normal locus, they may be designated as structures induced by the grafts. In contrast to those placed in the head, grafts in the pharynx induced the development of postcephalic outgrowths even in cases of complete union with the host. However, decapitation of the host at varying distances from the graft gave little evidence of graft dominance more than 2 mm. anterior to the level of implantation. Because of removal of the pharynges of the hosts, usually 3 or more weeks elapsed between the operation and the first feeding. During this time a tendency for the grafts to migrate was noted; and, accordingly, in certain instances longer periods of starvation were instituted, and cases of fusion of grafts with the host heads resulted. In both the pharynx and the tail regions the orientation of the implant had far less effect upon the final result than did the presence or absence of a

GRAFTS IN THE HEAD REGION

E. dorotocephala into *E. tigrina*.

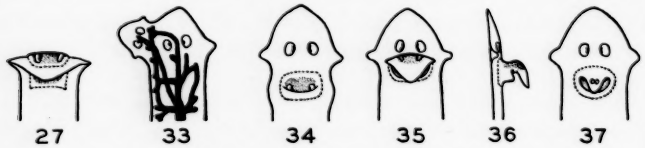


FIG. 27.—DVAA. Union complete. 7 days. New tissue around ventral surface of implant. Crawled with anterior portion of head raised. (Pl. I, FIG. 28.—Photograph of same animal 266 days later.)

GRAFTS IN THE ANTERIOR PREPHARYNGEAL REGION

FIG. 33.—DDAA. Anterior and right sides of graft failed to unite with host. 56 days. Graft larger than host head and controlled more posterior regions of host. Cephalic lobes, head shape, and epithelia of implant resembled those of host.

FIG. 34.—DDAP. Union complete. 21 days. New tissue partially covering anterior portion of implant. No appreciable change was observed during next 9 weeks.

FIGS. 35 (DORSAL VIEW) AND 36 (LATERAL VIEW).—DVAA. Union by posterior portion of dorsal surface only. 21 days. Short postcephalic outgrowth present. No appreciable change noted during next 9 weeks.

FIG. 37.—DVAA. Union complete. 21 days. Graft nearly inclosed by new tissue. Teratophthalmic outgrowth with dorsal epithelium of host and ventral epithelium of implant. No change noted during next 9 weeks.

free surface. For this reason the data in these sections will be described under two headings only.

Complete takes (61 cases).—Complete takes of cephalic grafts in the pharyngeal region generally induced tubular outgrowths which projected either from the dorsal or ventral surface of the host. (In 1 case no outgrowth developed, but in this it was noted from the first that the implant did not show the motor activity that was characteristic of all cephalic grafts.) When the eyes of the graft were subterminally located in the outgrowth, supernumerary photoreceptors developed at about the same level as those of the graft. This occurred even when the dorsal epithelium of the outgrowth was invaginated, as was the case when the outgrowth extended ventrally (Fig. 38). When the outgrowth developed at right angles to the anteroposterior axis of the graft, modification of the polarity of the graft was demonstrated by the development of eyes at right angles to

those of the implant and occasionally the complete resorption of the original eyes (Fig. 39). When the eyes of the implant were located terminally, they did not regress, and none were induced in the host (Fig. 40). These facts may be interpreted as follows. When there is no part of the outgrowth representing a more anterior level than that of the eye-level, the eyes of the graft take up a terminal position in the outgrowth. In this case the rest of the graft and the induced portions of the outgrowth which correspond to more posterior levels do not produce eyes. On the other hand, if the eyes were subterminal, the more anterior portion of the outgrowth produces, at a certain distance from the tip, the physiological level at which eyes can develop or persist. Since the outgrowth is tubular, the dominance extends radially, resulting in a ring of eyes which in these experiments varied from two to eight, depending upon the diameter of the outgrowth and the "scale of organization" (Child, 1931).

Figure 41 shows the only complete take in this region in which a pharynx developed anterior to the graft. This was located in reversed polarity and may be assumed to have been induced by the implant. It was considerably smaller than the one which appeared posterior to the graft. That there had been a complete reversal of polarity of this portion of the host was proved when there was no regeneration following the removal of the host head a short distance in front of the anterior pharynx.

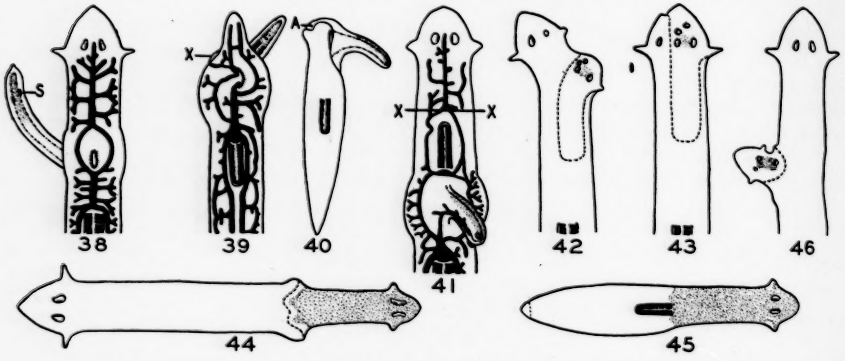
A gradual migration cephalad of grafts completely united with their hosts was noted in 9 cases. This was not as rapid as was observed in some cases of incomplete union, but in 1 case resulted eventually in fusion between the graft and host heads (Figs. 42 and 43). A rather significant feature of this migration was the disappearance of the cephalic lobe and eye of the host on the side nearest the graft. As the latter approached, these structures decreased in size and lost their pigmentation in a manner resembling stages in starvation. On the other hand, unpigmented new tissue was being laid down posterior to the graft, leaving a well-marked migration path behind. This indicated that host tissue was being used up anterior to the implant, while growth was taking place in the cells directly behind the graft. Consideration of these phenomena is reserved for the discussion.

In 8 cases grafts which united completely with the host were given a free surface. Four of these were given a free lateral and 4, a free posterior surface. In every case the anteroposterior axis of the resulting outgrowth was determined by the free surface and not by the original polarity of the graft. Figures 44 and 45 show a case in which a free posterior surface resulted in a 180° modification of graft polarity and the development of two additional eyes. These later fused with the original graft photoreceptors. Figure 46 shows a case in which a free lateral surface resulted in a modification of about 80° in the graft polarity.

In 3 cases the polarity was modified twice in the course of the observations. These were complete takes which were subsequently given a free lateral surface by a V-shaped cut in the side of the host. Figures 47 and 48 show the most clear-cut case. Freeing the lateral surface of the graft resulted in the development of a cephalic lobe at the anterior and another at the posterior margin of the graft and a photoreceptor beside the right eye of the graft (Fig. 47). These fused to form the right eye of the composite head. Meanwhile, as the graft migrated cephalad, the left side of the outgrowth fused with the right side of the host head. The right eye of the host and the two approximated cephalic lobes were resorbed (Fig. 48). Thus the polarity of the graft, after having been modified about 50°, finally, came to approximate its original condition.

Incomplete takes (52 cases).—The results of incomplete union differed from those of perfect takes in degree rather than in kind. In general the induced outgrowth of host tissue was greater and there was evidence of graft dominance extending a greater dis-

GRAFTS IN THE PHARYNGEAL REGION



FIGS. 38, 42, 43, 44, AND 45.—*E. tigrina* into *E. dorotocephala*. FIGS. 39 AND 46.—*E. tigrina novangliae* into *E. dorotocephala*. FIGS. 40 AND 41.—*E. dorotocephala* into *E. tigrina novangliae*. Note that in all cases the reconstituted pharynx is posterior to original pharynx level (level of implantation).

FIG. 38.—DDAA. Union complete. 42 days. Tubular outgrowth developed covered with ventral epithelium and containing two supernumerary eyes (*S*, only one shown).

FIG. 39.—DDAP. Union complete. 35 days. Original eyes disappeared, and four pigmented areas developed. Tail-like outgrowth developed from right side of host anterior to implant. Host, decapitated at *X* (about 2 mm. from implant) 1 week earlier, completely failed to regenerate.

FIG. 40.—DDAP. Union complete. 42 days. Large graft (1.4 × 1.1 mm.). Ventral view. Large tubular outgrowth has developed with terminally located eyes. Decapitated 4 weeks earlier about 3 mm. from graft. Regeneration limited to small triangle of tissue (*A*).

FIG. 41.—DDAP. Union complete. 28 days. Large graft (1.3 × 1.0 mm.). Pharynx with reversed polarity induced anterior to graft. Extent of graft dominance cephalad indicated by anterior reorganization of digestive tract. Host failed to regenerate after section at *X–X* 14 days later.

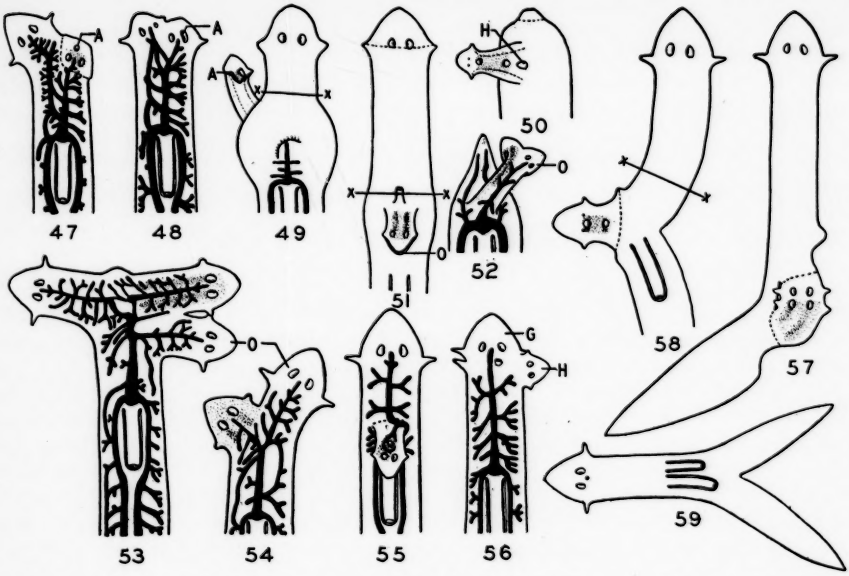
FIGS. 42 AND 43.—DDAA. Implanted to right of middle of host. Union complete. FIG. 42.—63 days. Path of graft migration indicated by new tissue. Nearest cephalic lobe and eye of host reduced in size with pigmented area entirely absent. Unpigmented outgrowth anterior to left graft eye. FIG. 43.—77 days. Entire right side of host head replaced by graft and surrounding tissues.

FIGS. 44 AND 45.—DDAA. Large graft (1.3 × 0.9 mm.). Union complete, but graft given a free posterior surface 7 days after implantation. FIG. 44.—31 days. Graft polarity modified 180°, and two pigmented areas developing behind original ones. Line between graft and host still distinct. FIG. 45.—74 days. Graft retained specific characters and reversed the polarity of a portion of host, as indicated by ciliary movement, orientation of pharynx, and regeneration of a tail after fission.

FIG. 46.—DDAA. Union nearly complete. 28 days. Left side of graft cut free from host 18 days after implantation. Left graft eye became right eye of outgrowth, and right graft eye eventually disappeared. Six weeks later graft had migrated cephalad, producing a Y-shaped compound resembling Figs. 54 and 61.

tance cephalad than in the case of complete takes. Of the four surfaces of the graft, the posterior united with the host most frequently (44 cases, 85 per cent).

In 13 cases the graft united by three of its four cut surfaces, and tubular outgrowths open at both ends resulted. In 4 of the foregoing the free surface was lateral. In 3 of



FIGS. 47, 48, 50–59.—*E. tigrina novangliae* into *E. dorotocephala*. FIG. 49.—*E. dorotocephala* into *E. tigrina novangliae*. FIGS. 49, 50, 57–59.—DDAA. FIGS. 51–56.—DDAP.

FIGS. 47 AND 48.—42 and 63 days, respectively. Union complete. FIG. 47.—V-shaped incision made in right side of host opposite graft 1 week earlier. Eye (*A*) has developed distal to left graft eye. Cephalic lobes developing anterior and posterior to implant. Modification of graft polarity about 50°. FIG. 48.—Graft and host heads fusing. Right eye and auricle of host regressing. Unpigmented area of supernumerary eye (*A*) fused with right graft eye. With resorption of right side of host head, fusion was complete and polarity of graft returned to approximately original condition.

FIG. 49.—Right anterior corner of graft failed to unite with host. 28 days. Tubular outgrowth covered with ventral epithelium. Eye (*A*) developed opposite left graft eye. Graft polarity modified about 40°. Host decapitated at *x*–*x* regenerated normal head in 2 weeks.

FIG. 50.—Part of left and right sides of graft failed to unite with host. 21 days. Small head with two pigment spots has developed from left side of graft. Eye beside hole (*H*) on right side did not regress during 7 weeks of observation. Regeneration of host inhibited.

FIGS. 51 AND 52.—14 and 35 days, respectively. Anterior surface of graft failed to unite with host. FIG. 51.—Small pharynx with reversed polarity induced anterior to graft. Head has developed from host portion of tubular outgrowth (*O*). Sectioned at *x*–*x*, tail with two-branched digestive tract developed (Fig. 52).

FIGS. 53 AND 54.—35 and 96 days, respectively. Anterior and part of lateral surfaces of graft failed to unite with host. Large graft (1.5 × 1.1 mm.). FIG. 53.—Head (*O*) regenerated from free host surface behind graft. Right side of host tore apart at level of implant. FIG. 54.—Host head removed 3 weeks earlier failed to regenerate. During following 8 weeks host epithelia completely replaced those of graft.

FIGS. 55 AND 56.—35 and 105 days, respectively. Right anterior edge of graft failed to unite with host. FIG. 55.—Graft polarity modified about 70°, and third eye has developed. FIG. 56.—Graft epithelia replaced, but implant (*G*) has migrated cephalad. Greatly reduced host head (*H*) disintegrated 1 week later.

FIG. 57.—Sides of graft failed to unite with host. 31 days. Portion of host lateral to implant tore apart. New tissue anterior to graft has developed eyes and auricles with reversed polarity.

FIGS. 58 AND 59.—21 and 49 days, respectively. Left side of graft failed to unite with host. FIG. 58.—Host lateral to graft tore apart 1 week earlier. Modification of graft polarity indicated by position of auricles and of two pigmented areas. Following decapitation at *x*–*x*, tail and second pharynx developed in host anterior to graft. FIG. 59.—Graft eyes reduced to pigmented areas only.

these cases a modification in polarity was indicated by the position of the regenerated eyes and cephalic lobes. In the fourth, in addition to a large part of the left side of the graft failing to unite, there was a small hole between the graft and host on the right (Fig. 50). The right graft eye close to the hole did not regress, as generally occurred when there was a modification of 90° in the graft polarity (compare with Figs. 46, 58, and 59).

The 5 cases in which the anterior surface of the graft was free developed larger outgrowths than did the 3 cases mentioned above. In 3 of the 5 cases the outgrowths were ventral (Fig. 49); in 2, dorsal. In 2 cases there was a slight modification of polarity, and in 3 the free surface was approximately parallel with the eyes and polarity remained unchanged.

In 4 cases with reversed anteroposterior axis, in addition to the anterior surface of the graft, a small portion of each lateral surface failed to unite. In each case two cephalic lobes were regenerated and a tubular postcephalic outgrowth was induced. From the free surface at the posterior edge of the host window a secondary head developed. In 1 case (Figs. 51 and 52) a very small but functional pharynx developed in reverse orientation just anterior to the transplant. Following decapitation, a tail with the characteristic two-branched digestive tract developed from the anterior cut surface. In the second case the graft and secondary head tore through the right side of the host and thus obtained access to the substrate. During the next 3 weeks they were almost constantly pulling against the host head, taking on the appearance of the letter *T* (Figs. 53 and 54). Great difficulty was experienced in inducing this specimen to feed. During 4 weeks of starvation subsequent to the condition shown in Figure 53, the original host head was reduced to a size about equal to that of the graft head. In an attempt to prevent the excessive stimulation of three heads in the presence of food, the original head of the host was removed at this time. The cut surface healed, and the two remaining heads took up an anterior position. However, it was not until 3 weeks later that, as a result of being placed in the dark at feeding time (cf. above, p. 218), the specimen began taking food again. At this time it was only 1.6 mm. long, and the secondary host head was larger than that of the graft (Fig. 54). As it increased in size, the graft head grew more rapidly, until it again became equivalent to that of the host. During the 7 weeks of complete starvation the line of demarcation between graft and host remained quite distinct. However, as the specimen increased in size, the graft epidermis was observed to fade into host tissue along the line of separation until eventually the photoreceptors, head shape, and cephalic lobes resembled those of the host. This situation will be treated in the discussion.

In the third case, only a small portion of the right side of the graft was free, while a considerable portion of the left did not unite with the host. During regeneration of the graft a third eye appeared anterior to the left eye, and cephalic lobes developed opposite it and the right eye of the graft (Figs. 55 and 56). The implant increased in size as it migrated cephalad, and eventually dominated the host, while the host head became merely an appendage (Fig. 56). The fourth resembled the third except that more of the right side of the graft was free and there was no alteration of graft polarity. As a result of failure of the digestive tract of the graft to unite with a main branch of that of the host, the graft and the secondary host head received insufficient food and eventually regressed.

Two of the implants united by their anterior and posterior surfaces, leaving the larger portion of the lateral surfaces free. In both cases the eyes developed in the portion of

the host in contact with the anterior part of the graft, i.e., in reversed orientation. In one, two structures resembling cephalic lobes developed in host tissue lateral to the induced eyes (Fig. 57). These appear to be another expression of the same situation observed in the development of photoreceptors in the tubular outgrowths resulting from complete takes. It is evident that the distance from the anterior edge of the graft to the graft eyes in one direction and to the induced eyes in the other was approximately equal. If the two lateral surfaces of the graft and host were fused, a situation resembling that in Figure 77 would result.

There was only 1 case in which a graft united with the host by one of the lateral surfaces. Two weeks after implantation it tore through the tissue on the left side of the host. The next week two pigment spots appeared distal to the left one of the graft, and a pharynx developed posterior to the level of implantation (Fig. 58). At this time the host head was removed. Instead of a head, a tail developed from the cut surface, and later a second pharynx appeared in reversed polarity (Fig. 59).

In 30 cases only the posterior surface of the graft united with the host. Figures 60 and 61 represent the typical result in this group. By an increase in size of the graft and a decrease in size of the host prepharyngeal region a Y-shaped compound resulted.

Five hosts tore apart either anterior or posterior to the implant. When the graft remained attached to the posterior piece, there was no regeneration of the host (Fig. 63). In these the graft formed the anterior portion of the worm. When the graft was attached to the anterior half, one or two tails developed from the exposed surfaces of the host. However, no pharynges developed, and the specimens gradually decreased in size.

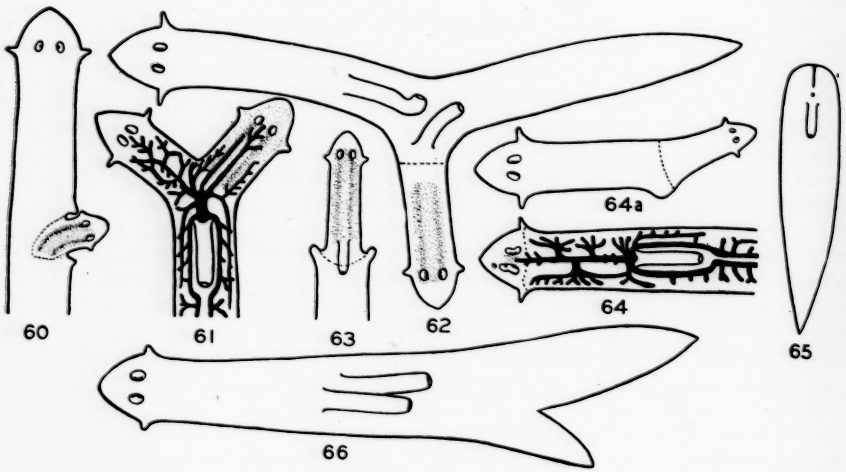
In 5 cases the windows were made by removing the dorsal wall and the underlying mesodermal tissues without cutting through the ventral wall. Grafts placed in these united with the dorsal surface of the host only. In addition there were 3 cases in which, although the usual procedure was followed, the ventral surface was not involved in the union. All 8 examples regenerated the triangular head shape and cephalic lobes but within 2 or 3 weeks became greatly reduced in size and showed evidences of lack of nourishment. When fed blood, it could be seen that no connection had been established between the digestive systems of graft and host.

Grafts placed in reversed dorsoventral orientation usually united with the host by their posterior surfaces, with the anterior tip of the graft bending back upon itself. When union of digestive tracts of graft and host occurred, the grafts persisted; otherwise they decreased in size and were lost.

Removal of the host head.—In 45 cases the host was decapitated 2 weeks or more after transplantation. Thirty were cut close to the graft (i.e., about 1–2 mm. anterior to it). In 18 of these regeneration of another host head was completely prevented (Figs. 39, 40, 50, 52). In the other 12 cases a normal head regenerated, but in 5 of these the grafts were regressing at the time of decapitation and so might perhaps be excluded from consideration. In 7 cases the section was made about 3 or 4 mm. from the graft. Five of these regenerated normal heads, and in only 2 cases was host regeneration inhibited (Fig. 59). In 8 cases the host was decapitated a short distance posterior to the host head. The only specimen that did not regenerate a normal head was one mentioned earlier (Fig. 41) in which the graft had induced a pharynx in reversed polarity anterior to the level of implantation. All the controls regenerated normal heads. It is evident that dominance of cephalic grafts does not usually extend much more than 2 mm. cephalad in large planarians such as were used in these experiments.

In 3 cases removal of the host body anterior to the graft, by section as near as possible to the graft, resulted in development of bipolars with a small but normal head instead of a tail at the posterior surface of the piece removed (Figs. 64, 64*a*). In these the polarity reversal was so firmly established that removal of the dominant region under which it had developed did not destroy it.

GRAFTS IN THE PHARYNGEAL REGION



FIGS. 60–64.—*E. tigrina novangliae* into *E. dorotocephala*. DDAA. FIG. 65.—*E. dorotocephala*, control. FIG. 66.—*E. tigrina novangliae*, control.

FIGS. 60 AND 61.—Anterior surface of implant failed to unite with host and right side of host tore apart at level of implantation. FIG. 60.—14 days. Graft has regenerated cephalic lobes. FIG. 61.—42 days. Graft has increased in relative size and induced a pharynx. During next 9 weeks the two heads remained approximately equivalent.

FIG. 62.—Graft united to host by posterior surface. 35 days. The only case in which a pharynx with normal polarity appeared anterior to level of implantation.

FIG. 63.—Graft united by posterior surface. 14 days. Host tore apart anterior to implant 1 week earlier. No regeneration from exposed surfaces of host.

FIGS. 64 AND 64*a*.—Union complete. 35 days. 21 days after subsequent section of host just cephalad to implantation level. FIG. 64*a*.—Anterior part of host has regenerated a head on posterior surface. FIG. 64.—Posterior part of host containing implant has developed cephalic lobes. Two supernumerary eyes have appeared anterior to left graft eye.

FIG. 65.—Control. 7 days. Lateral margins of window united and animal tore apart. Posterior portion of animal developed single pigment spot and small pharynx but refused food.

FIG. 66.—Control. 63 days. Window in host remained open for more than a week. Incision made in right side 4 weeks after operation. Three weeks later tail-like projection appeared. Second pharynx appeared 2 weeks later.

Controls.—With only a few exceptions the tissue which was removed was replaced in the controls and a functional pharynx regenerated. In 1 case the window did not fill in completely, and a small head regenerated from its posterior edge. In another the two

sides fused together, and, when the specimen tore apart subsequently, there was no regeneration at the anterior surface. A single median eye and a pharynx developed, but the piece would not feed (Fig. 65). In several controls in which there was little tissue along the sides of the window, the worms tore apart at this point. Later a head regenerated on the posterior piece and a tail on the anterior. In 3 cases after healing, tail-like outgrowths developed behind the level of operation. Figure 66 shows a case in which a pharynx developed in association with such an outgrowth.

GRAFTS IN THE TAIL (174 CASES)

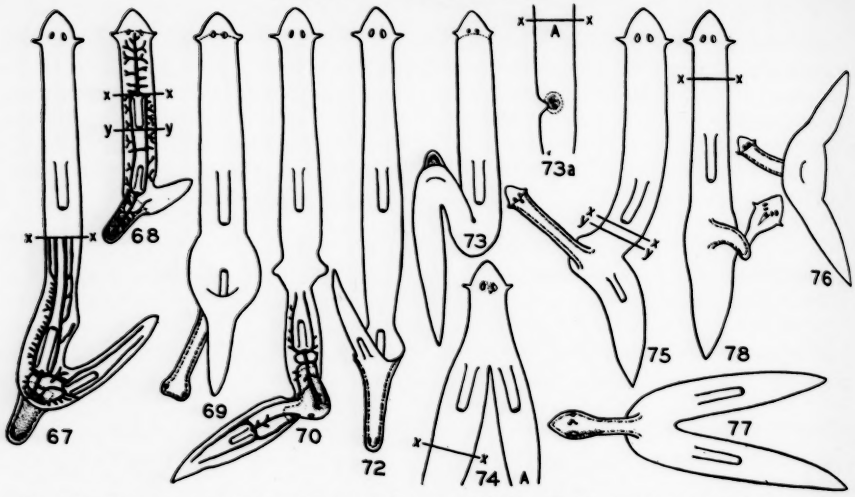
The tail proved to be the most labile of the regions studied. Cephalic grafts placed in this region induced the greatest outgrowths and produced the greatest reorganization of the host. Pharynges were induced equally well anterior or posterior to the level of implantation. They developed about the same distance anterior or posterior to the graft, but their lengths and diameters were proportional to the amount of tissue in which they developed. There were a few cases in which, in addition to a pharynx, a tail was induced anterior to the graft. Data on the range of dominance anteriorly showed that, unless the host was unusually long, the inhibitory power of the graft extended about to the fission zone of the host and occasionally into the pharyngeal level itself.

When the two-tailed forms produced by removal of the host heads were followed for long periods (6 months to a year), they were observed to fuse along the adjoining lateral margins, eventually producing double monsters. The fission products of these forms usually developed three or four eyes, two pharynges, and a more or less completely doubled digestive tract.

A few triangular implants containing chiefly ganglionic material were placed in the tail. Although containing less tissue than the rectangular grafts used generally in this study, they showed greater inductive capacities than the latter. No tendency to migrate was observed in implants in this level.

Complete takes (71 cases).—Cephalic grafts in the tail which united completely with their hosts produced the same type of tubular outgrowths as did complete takes in the pharynx. When the photoreceptors came to lie in the terminal portion of the outgrowth (43 cases), there was no alteration of graft polarity (Figs. 67, 68, and 69). When subterminal, supernumerary eyes developed in the induced tissue (Fig. 70). These outgrowths generally were larger than those resulting from grafts in the pharynx level (Pl. I, Fig. 71). Also, it usually was possible to observe that control by the graft extended some distance anterior to the level of implantation.

Pharynges were induced in the host in 19 cases. Of these, 13 developed two supernumerary pharynges, one posterior to the graft in normal orientation and the other anterior to it with reversed anteroposterior axis (Figs. 67, 70, and 72). In 4 of the 6 cases in which there was but one induced pharynx, it was posterior to the graft, while in the other 2 it was anterior (Fig. 69). In the latter the absence of a posterior pharynx may have been the result either of the small amount of host tissue posterior to the graft or of the proximity of the posterior tip of the tail, or both. In this connection it should be mentioned that there appeared to be a relationship between the length and diameter of the pharynx and the amount of tissue in which it developed, but the level at which it appeared was determined by the implant. Figures 67, 70, and 72 illustrate this situation. In these, both induced pharynges developed at approximately the same distance from the graft; but the posterior one, extending into the narrow tip of the tail, was both



FIGS. 67, 68, 70, 72, 75, 76, AND 78.—*E. tigrina novangliae* into *E. dorotocephala*. FIG. 69.—*E. dorotocephala* into *E. tigrina novangliae*. FIGS. 73 AND 74.—*E. tigrina* into *E. dorotocephala*.

FIGS. 67 AND 68.—DDAP. Union complete. 21 days. Host fissioned anterior to implant and normal head and pharynx reconstituted.

FIG. 67.—Digestive tract reorganized and pharynx developed anterior, as well as posterior, to implant. Note that reversed pharynx is larger (also in Figs. 70 and 72). Following section at *x–x*, tail regenerated on posterior piece.

FIG. 68.—Digestive tract reorganized, and pharynx developed anterior to implant. Digestive tract was not seen in tip of tail, since no food entered. Normal head regenerated following decapitation at *x–x*. Tail developed following section at *y–y* 3 weeks later.

FIG. 69.—DDAA. Union complete. Graft has induced tubular outgrowth and pharynx with reversed polarity.

FIG. 70.—DDAP. Union complete. 14 days. Digestive tract reorganized and pharynges developed anterior and posterior to implant. Two eyes (only one figured) in new tissue posterior to graft.

FIG. 72.—Implants placed in both head and tail. DDAA. Union complete. 35 days. Graft in head has been incorporated into host. Pharynges have developed anterior and posterior to ventrally projecting tubular outgrowth induced by second graft.

FIGS. 73 AND 74.—Union complete. DDAA. 14 and 35 days, respectively. FIG. 73.—Ventrally projecting tubular outgrowth has developed and posterior part of host is under graft control. FIG. 73*a*.—Portion of animal after V-shaped incision was made, giving graft a free surface. One week later host fissioned at *x–x*. FIG. 74.—Original eyes reduced to pigmented spots. New eyes, cephalic lobes, and two pharynges have developed. Tail, instead of head, has regenerated at *A*. One week later animal fissioned at *x–x*.

FIG. 75.—DDAA. Left and posterior edges of graft failed to unite with host. 28 days. Graft polarity modified about 120°, and new eye has developed. Digestive tract (not shown) has reorganized. Tubular outgrowth and pharynx have developed posterior to implant. No regeneration occurred when sectioned at *x–x* 4 weeks later. After second removal (*y–y*), 8 weeks later, tail regenerated and second pharynx with reversed polarity developed anterior to implant.

FIG. 76.—DDAA. Anterior surface of implant failed to unite with host. 21 days. Tail, instead of head, regenerated on host following fission 1 week after implantation. Graft has induced a tubular outgrowth lined with dorsal epithelium.

FIG. 77.—DDAA. Very small part of right surface of graft failed to unite with host. 35 days. Implant induced tubular outgrowth with two eyes (one not shown) opposite those of graft and two pharynges. From hole two auricle-like structures developed. Anophthalmic head developed after fission of host 5 weeks earlier. When removed after 2 weeks, a taillike structure regenerated.

FIG. 78.—Triangular graft DDAA. Right side of graft and very small part of left failed to unite with host, leaving a hole on left. 56 days. Tubular outgrowth developed posterior to graft. Second pair of eyes developed between hole and lateral surface of outgrowth. Host regenerated normal head after section at *x–x*.

shorter and smaller than the anterior. Some of Santos' figures (Figs. 26, 29, 36, and 37) show the same condition, but he does not comment on the situation.

In 5 cases the host tissue on one side of the graft was removed, thus giving the graft a free surface. All 5 regenerated cephalic lobes and one or two eyes; and in each case the final polarity of the outgrowth was determined by the free surface (Figs. 73, 73*a*, and 74). There was no indication in these of migration such as was described for grafts in the pharynx.

Incomplete takes (103 cases).—Grafts in the tail which did not unite completely with their hosts regenerated their missing parts more or less completely, depending upon the amount of free surface. When union was nearly complete, tubular outgrowths developed; but when one or more sides of the graft remained free, the resulting outgrowth was flat. As was true for other levels, the polarity of the outgrowth was determined by the free surface. This was shown by the cases in which it did not coincide with the original anterior surface of the graft (Fig. 75).

In 16 cases union was nearly complete, and a tubular outgrowth developed. In 14 of these the outgrowth extended dorsally with the dorsal surface of the graft and host outside (Fig. 75). In the other 2 cases it was ventral with the dorsal surface inside (Fig. 76).

In 5 cases a very small portion of one edge of the graft did not unite, resulting in a hole between the graft and host. One or two cephalic lobes regenerated from this; and in the cases in which union was otherwise complete, a pair of eyes developed in the host portion of the outgrowth at the same level as those of the implant (Fig. 77). In 3 instances in which the graft united by only one surface, a second pair of photoreceptors developed between the hole and the lateral surface of the outgrowth, resulting in a compound head with three cephalic lobes and four photoreceptors (Figs. 78 and 79). These cases resemble rather closely that shown in Figure 57, in which a pair of eyes and two cephalic lobes developed in the new tissue anterior to an implant in the pharyngeal region. In 1 case a graft eye close to such a hole persisted for 14 weeks in a distinctly ectopic position, whereas usually photoreceptors at such a distance from the normal locus disappeared completely in 4–6 weeks (Fig. 82).

When the grafts in this level united by one edge, it was commonly the posterior (36 cases). In these there was the usual regeneration of missing parts by the graft head and reorganization of the host both anterior and posterior to the level of implantation. Since cases similar to those obtained in this study have been described and figured by Santos (1931), they will not be taken up in any detail. In 4 cases a lateral surface of the graft united with the host. In these, as with some of the foregoing, in which a portion of a lateral, as well as the posterior, surface was involved in the union, there was the usual modification in the polarity of the implant.

In a number of cases there were indications of the development of a tail between the host pharynx and the graft-induced one. These appeared first as slight swellings, which, as they grew, became supplied with a two-branched digestive tract. There were varying degrees of development of these outgrowths from slight projections to well developed tails. Figure 83 (Pl. I) is a photograph of one in which the supernumerary tail was well developed.

As was the case in other levels, reversal of the dorsoventral axis resulted in a proliferation of tissue at the line of union between graft and host. There was a tendency for the union to be superficial, involving only the two dorsal surfaces (Pl. I, Fig. 84). This was probably a result of the greater contraction of the ventral than the dorsal sur-

face of the grafts when they were inverted. Incomplete unions were very common in these grafts, and the host window often tore apart directly anterior or posterior to the graft. In Figure 85 (Pl. I) we see a case in which the two portions of host tissue lateral to the graft developed into tails after the original host tail had torn off. However, when both dorsal and ventral surfaces united, there often was regeneration of one or two heads and tails. In one of these the dorsal surface of the host was united with the ventral surface of the graft, and in the other the ventral surface of the host with the dorsal surface of the graft. Figure 86 (Pl. I) shows an interesting example of this sort. In this case the host underwent fission directly behind the implant. A host head developed with reversed polarity, apparently induced by the graft, and with its ventral surface continuous with that of the graft. A single functional pharynx was induced, probably by the combined dominance of the two heads.

Cephalic grafts in the tail with the anteroposterior axis of the graft reversed retained their polarity in cases of complete union and reorganized the host equally well as did grafts in normal orientation. With reversed anteroposterior orientation, however, the graft formed the anterior, rather than the posterior, half of the outgrowth; and the induced eyes were posterior, rather than anterior, to the graft (Figs. 68 and 70).

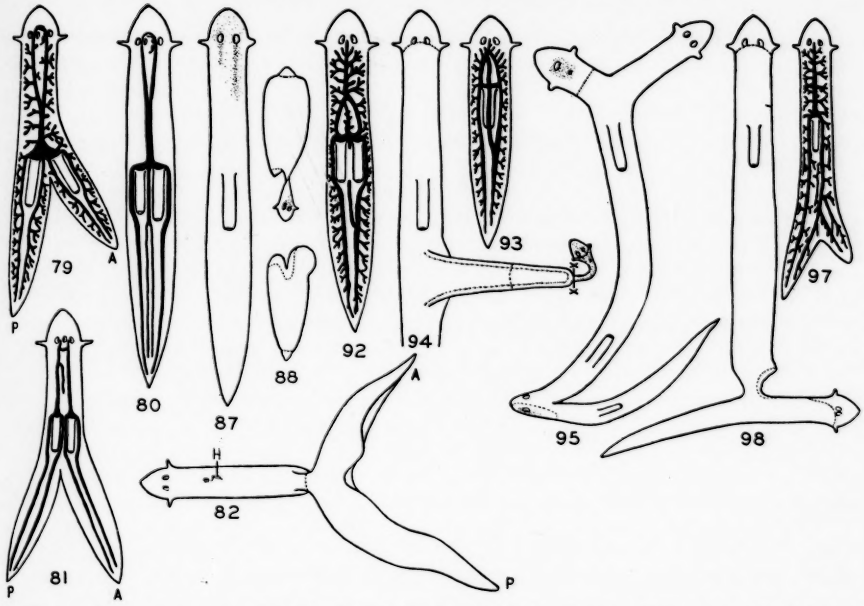
In cases of incomplete union, cephalic grafts in the tail with anteroposterior axis reversed gave results indistinguishable from those whose anteroposterior axes coincided with those of the hosts.

In 17 cases the host tore apart immediately anterior to the graft. In 14 of these the graft completely prevented regeneration from the exposed surfaces of the host. In the other 3 a tail, instead of a head, developed in the host. It was in grafts such as these that the persistence of the graft species characters was most marked. Figure 87 shows a case, 20 weeks after grafting, in which the species differences still were distinctly recognizable. The line between graft and host had faded out, but it was evident that the anterior and right portions of the compound were *E. tigrina* tissue while the posterior and left were *E. dorotocephala*. The persistence and replacement of graft tissues will be treated under "Discussion."

In 6 cases the posterior part of the host window tore apart, leaving the graft attached to the anterior piece of the worm. From the torn edges two tails developed in 1 case (Pl. I, Fig. 85), one tail in 3 cases (Pl. I, Figs. 89 and 90), and there was no regeneration in 2 cases (Fig. 88).

In 5 cases a pharynx was induced with reversed polarity. These grafts may be contrasted with similar ones in the pharyngeal region in which induction of a pharynx did not occur. In the latter the distance from the graft to host head was so short that there was no level of the gradient between them at which a pharynx could develop. However, in the case of implants in the tail the tissue between the two heads included the pharynx level, and reorganization by the graft expressed itself in the induction of a pharynx with reversed polarity.

In 40 cases the host head and part of the host body were removed 2 or more weeks subsequent to grafting. Of these, 10 were cut a short distance behind the host head, 10 about midway between the host head and the pharynx, 10 across the middle of the host pharynx, and 10 posterior to the pharynx. All of those cut close to the host head, and those cut in the prepharyngeal region regenerated normal heads anteriorly. Of those cut through the middle of the pharyngeal region, 4 regenerated normal heads and 6 developed tails, while all of those cut between the host pharynx and the graft regenerated



FIGS. 79–81, 92, AND 93.—*E. tigrina* into *E. dorotocephala*. FIGS. 82, 87, 88, AND 94.—*E. tigrina novangliae* into *E. dorotocephala*. FIG. 95.—*E. dorotocephala* into *E. tigrina novangliae*. FIGS. 79–82, 87, 92–95.—DDAA. FIG. 88.—DVAA.

FIGS. 79–81.—133 and 216 days, respectively. Left and a part of right side of graft failed to unite with host. FIG. 79.—Short outgrowth of tissue with two auricle-like structures developed from hole on right of graft. Tail, instead of head, regenerated from anterior surface (*A*) following fission. Fusion between two tails (*A* and *P*) beginning. FIG. 80.—Only main branches of digestive tract are figured. Lateral fusion of tails has resulted in doubled postpharyngeal region. FIG. 81.—Fission piece of Fig. 80. Note presence of three eyes and completely doubled digestive tract.

FIG. 82.—Triangular graft. 35 days. Right and very small part of left side of graft failed to unite with host. Left graft eye, close to hole (*H*), remained 14 weeks before final disappearance. Replacement of graft epithelia almost complete. Tail has regenerated from anterior end of host (*A*) following fission.

FIG. 87.—Union by posterior surface only. Host tore apart at implantation level 1 week after operation. 119 days. Graft has retained its specific characters, although line of union is no longer distinct.

FIG. 88.—Triangular graft. Posterior surface of graft failed to unite with host. 14 days. Graft polarity modified about 140°. Host fissioned anterior to implant and tore apart posterior to it. Graft inhibited anterior regeneration and dominated behavior of piece but was lost 2 weeks later.

FIGS. 92 AND 93.—Anterior surface of graft failed to unite with host. 182 days. FIG. 92.—Regenerated posterior third of fission piece with doubled digestive tract. Note two pharynges and partially doubled digestive tract. FIG. 93.—Piece fissioned from Fig. 92. Note single pharynx.

FIG. 94.—Triangular graft. Union by posterior surface of implant to dorsal surface only of host. 21 days. Original eye reduced to pigment spot, and two new ones have developed. Note long tubular outgrowth from host and small size of graft. Graft gradually decreased in size and eventually pinched off at *X–X* 6 weeks later. Induced outgrowth remained until termination of experiment on 105th day.

FIG. 95.—Double graft. Anterior implant in pharyngeal region, posterior in tail. Anterior united by right side only, posterior united completely. 21 days. Note three regions of influence in host: anterior under control of host head, middle under control of first graft, and tail dominated by second. Two weeks later, left eye of first graft was double, indicating 90° modification of polarity.

FIG. 97.—Control. *E. dorotocephala*. 42 days. Fission occurred at level of window; and, following, regeneration of normal head, outgrowth containing two-branched digestive tract appeared.

FIG. 98.—*E. dorotocephala*. Control for Fig. 73. 21 days. Right side of host window tore apart, and teratophthalmic head regenerated.

tails. In addition to this experiment, there were 58 cases of fission posterior to the pharynx but anterior to the implant. Of these, 33 developed tails anteriorly, 20 showed no outgrowth of tissue at all, and 17 regenerated heads. Some of the fissions here recorded occurred during the first week after operation. Of these, 16 developed normal heads, leaving only 1 case in which a normal head developed after the reorganization effects had begun to appear.

In all cases in which tails were induced, there was a tendency for the two tails to become parallel and fuse (Pl. I, Fig. 91). In those which were observed for long periods of time (up to 15 months) the two tails gradually approximated, until finally a worm with doubled pharyngeal and postpharyngeal regions resulted. When fission of this double animal occurred, the fission pieces usually developed two to four photoreceptors and a more or less completely doubled digestive tract, consisting, in the most extreme cases, of two anterior branches, two pharynges, and four posterior branches (Figs. 80 and 81). In 1 instance sixteen successive fission pieces from a single graft showed this condition. Sonneborn (1930) produced doubled forms of *Stenostomum* as a result of treatment with lead acetate. These abnormalities were passed from one generation to another by fission, and therefore he considers them to be hereditary modifications. From the same point of view, the modifications described above could be considered hereditary, since they depended upon a self-perpetuating pattern persisting from parent to offspring. However, it appears to the writer that a most important feature of this "inheritance" has not been taken into account. This is the fact that all the "offspring" resulting from fission are, in reality, parts of the parent and must necessarily partake of such axial modifications of the parent as the doubling described by Sonneborn. In the cases reported here, it is extremely improbable that there was any modification of the hereditary units which might persist through sexual reproduction. An experiment was performed with the planarians which, because of the very small number of animals, was inconclusive except in one respect. Two large double specimens of *E. dorotocephala* were cut transversely into three approximately equal pieces and allowed to regenerate. The two anterior pieces regenerated a tail and a single pharynx. The two middle pieces, each containing two pharynges, regenerated a normal head and a tail containing a three-branched digestive tract. The posterior pieces regenerated normal heads and partially doubled digestive tracts, containing two pharynges and three posterior branches (Fig. 92). During the second week of regeneration fission occurred in the posterior pieces, and the fission pieces both reconstituted a single pharynx and almost normal posterior intestinal branches (Fig. 93). Further experiments along this line are necessary before the factors involved can be determined. However, since the same animal could produce single as well as double individuals, the persistence of the latter condition could hardly be called hereditary, even in the sense of Sonneborn.

Triangular grafts (33 cases).—The first grafts which were made in this study were triangular grafts in the tail region, made according to the method of Santos (1931). These implants contained less tissue than the rectangular grafts which were used later, but contained more of the cephalic ganglia. The postcephalic outgrowths induced by these were generally larger than those of the rectangular grafts, but often the host digestive tract did not unite with those of the grafts, and the latter did not persist (Fig. 94).

In only 3 of the 33 cases of triangular grafts did the ventral surface of the graft unite with that of the host; in the other 30 cases it united with the dorsal surface of the host and the host ventral surface healed over. As a result of this, tubular outgrowths which

ended blindly were induced even in cases of incomplete union (Figs. 78 and 94), and the frequency of union between graft and host digestive tract was very much less than in rectangular implants. Among the rectangular grafts there were only 3 cases in which the ventral surface of the host did not unite with some part of the graft.

Double grafts (18 cases).—The effect of the level in which a graft is placed was strikingly illustrated in a series of 18 double grafts. Figure 72 shows the results of simultaneous implantation of cephalic grafts in the head and in the tail of the host. Both were complete takes; but the graft in the head healed without leaving any traces, while that in the tail induced a tubular postcephalic outgrowth and two pharynges.

Figure 95 illustrates the result of simultaneous transplantation in the pharynx and tail. In this case the anterior graft united by the right side only, while the posterior united by all surfaces. Three well-marked regions of influence can be seen. That controlled by the host head extended about to the level of implantation of the first graft. The first graft controlled the movements of the host a short distance anterior to it and posteriorly about half the distance to the pharynx induced by the second graft. The second graft controlled about equal distances anteriorly and posteriorly. Figure 96 (Pl. I) is a photograph of a case in which grafts placed in the pharynx and tail both united with the host by all four surfaces. The three regions of influence evident in Figure 95 can also be seen in this illustration.

Controls.—The large majority of the controls replaced the parts removed, and within 2–4 weeks were normal again. The exceptions consisted chiefly of cases in which the lateral edges of the window united, effectively preventing food from reaching the tip of the tail, which eventually either tore apart or disintegrated. In two cases a tail-like outgrowth developed behind the level of operation, and one of these was observed to be supplied by two branches of the digestive tract (Fig. 97). In one case one side of the window tore apart, and from it a teratophthalmic head regenerated. This dominated the behavior of the more posterior part of the specimen and completely reorganized it (Fig. 98).

DISCUSSION

TRIANGULAR VERSUS RECTANGULAR GRAFTS

The increased frequency of takes by using rectangular, instead of triangular, grafts was mentioned in the section on "Methods." In addition to this, the use of rectangular grafts has clarified the problem of polarity of the implants. In cases where implant and host united completely, Santos (1931) shows figures of small monophthalmic outgrowths of undetermined polarity and states that they were the usual result. In the experiments here, such outgrowths were common in triangular implants but were not observed in rectangular grafts. The small outgrowth of tissue in the triangular type grafts is thought to be the result of union of the grafts with the dorsal surface only of the hosts, since it was also observed in cases of incomplete union in which the host ventral surface was not involved. The monophthalmic condition was found by the writer to be the result of the partial removal of one of the graft eyes during operation and the absence of any regeneration. With rectangular grafts the ability of complete takes to reorganize host tissues was in many cases equal to that of incomplete takes. Pharynges were induced posterior to the implant in grafts in the pharynx; and in the tail, induction by complete takes extended both anteriorly and posteriorly (Figs. 39, 40, 41, 67, 68, 70, 72, and 96). In fact, 1 of the

2 cases in which a pharynx was induced anterior to a graft in the pharynx level was a case of complete union (Fig. 41). This is in contrast to the findings of Santos (1931) with triangular grafts.

POLARITY

The anteroposterior polarity of cephalic grafts is shown in these experiments to be extremely labile. In cases of complete union there was little evidence that the axes of the grafts were modified except in anteroposterior grafts in the head. In these it was not possible to determine positively whether the implant retained its original axial orientation or not, but it seems likely that reversal occurred. There was no indication of lack of co-ordination of movement between host and implant or of cilia beating in opposite directions. The direction of the ciliary beat has been used by Rustia (1925) and by F. S. Miller (1937) as a criterion of the polarity of planarians and seems to be reliable.

Grafts containing any appreciable amount of free surface always showed corresponding modifications of polarity. These modifications ranged from very slight changes, in which a single photoreceptor developed in close proximity to one of the graft eyes, through cases of 90° modification with two photoreceptors developing at right angles to the original ones (Figs. 46, 47, 48, 55, 56, 58, and 59), to complete reversal in cases in which the posterior surface of the graft remained free (Figs. 30 and 31). The latter type of modification was rare because the posterior surface of the grant united more commonly than any other, but the results were consistent.

The lability of the axial organization of the most complex and highly differentiated region of a planarian is quite remarkable. Under certain conditions eyes appear, and under others they regress and disappear. On the other hand, polarity varies with the position of the free surface or surfaces. These conditions are very difficult to reconcile with a concept of polarity such as that of Harrison (1921, 1936), involving models used in stereochemistry. In order to do so, it would be necessary to develop additional theories to explain the reorientation of the hypothetical molecules with each modification in polarity. In a case such as is shown in Figures 47 and 48, three such modifications must be accounted for. In addition, the correlation between the exposed free surface and the polarity of a cephalic graft is unaccounted for by such a theory. Similar difficulties are encountered in attempting to interpret the observed modifications of polarity in these experiments with the space-lattice concept of Przibram (1921). On the other hand, the theories of Goetsch (1929) seem to be, in reality, chiefly a restatement of observed conditions rather than any constructive hypothesis which may aid in a true understanding of the significance of this general feature of organisms.

By contrast with the foregoing, the gradient concept which has a large body of experimental proof requires no additional hypothesis to account for the determination of polarity by a free surface. According to this concept, polarity is a function of the presence of a dominant region and its morphogenetic activities along the anteroposterior axis. One of the most characteristic features of the dominant region is its relatively high rate of physiological activity, and so the problem of the origin of polarity resolves itself into the origin of a region of great activity. That regenerative processes are characterized by increased physiological activity of the cells at the free surface has been demonstrated in a large number of forms. That the polarity of a planarian regenerate is independent of the nervous system of the piece is indicated in the histological studies by Flexner (1898) on *P. torva* (syn. *E. tigrina novangliae*) and by Keiller (1910) on *Planaria*

simplicissima (syn. *Curtisia foremanii*), which showed that the ganglia of the regenerate develop independently of the old nervous system. Similarly, Beyer and Child (1930) concluded from experiments on the reconstitution of lateral pieces of *E. dorotocephala* that the nervous system is not an essential factor in determining the polarity of the regenerate.

Accordingly, we may suggest that the changes in polarity of the grafts resulted from activation of the cells of the free surface, which set up a new dominant region in the regenerate, and that this region gradually expanded its physiological and morphogenetic control until the entire graft-controlled tissue was repolarized. There has been little work throwing light on gradient conditions within the head, but it is possible that the slope here is very slight. The great lability of the polarity of cephalic grafts would seem to suggest this.

As was also found by L. V. Morgan (1906) and Santos (1931), the dorsoventral organization of the planarian pieces used in these experiments was not susceptible to modification by any of the situations which arose as a result of grafting. In every case the dorsal and ventral epithelia of grafts retained their peculiarities, even though, as time passed, they gradually became replaced by host tissues.

The results of these grafting experiments emphasize the fact that the tail of planarians which undergo fission is physiologically quite different from the head. It has been recognized for some time (Hyman, 1916) that the posterior, growing region of certain forms involves special conditions within the primary anteroposterior gradient. That this region is definitely polarized is shown by the fact that tails are regenerated from posterior surfaces of pieces from this level and heads always appear at the anterior surfaces. However, the experiments reported here, and those of Santos (1931) and of F. S. Miller (1937), indicate that polarization is by no means as strong posterior to the pharynx in *E. dorotocephala* and *E. tigrina* as in more anterior levels, and emphasize the fact that the high physiological activity and great regenerative powers of this region should be more thoroughly investigated from a gradient point of view.

REPLACEMENT OF GRAFT BY HOST TISSUE

With few exceptions there was a gradual replacement of graft epithelium by that of the host. This has been described on page 219; and it was noted that the photoreceptors, as well as the epithelia, were replaced. Since the eyes are a part of the nervous system, this fact would suggest that the other tissues of the graft, as well as those which could be observed, probably were also involved in the replacement.

The replacement of tissues was especially rapid in small grafts which were composed largely of ganglionic tissue. On the other hand, large grafts which contained, in addition to approximately the same amount of nervous tissue, some prepharyngeal tissue retained their specific characters for a considerable longer period, and in some cases did not show any appreciable replacement of epidermal structures during the entire course of the observations. Even in these, however, the line between graft and host faded out in time and the tissues of that region took on an appearance which was intermediate between the two species.

The structure of the anterior part of a planarian seems to offer a suggestion as to the factors which determine whether a heteroplastic graft will or will not retain its specific differences. The head of a planarian consists largely of nervous and muscular elements

with very little parenchymatous tissue. It is from the latter that the more specialized cells are normally differentiated. By contrast, in the anterior prepharyngeal region nervous tissue is limited chiefly to the nerve cords; and, although the diverticula of the digestive tract occupy a much larger space than in the head, there is considerably more packing or parenchyma tissue. Curtis and Schultze (1934) have found also that there are more "formative cells" in this than in the ganglionic region.

Grafts which retained their specific characters all united with the host by only a portion of the cut surfaces. In 2 cases they were grafts in reversed dorsoventral orientation (Fig. 29), and in the others they were grafts in normal orientation which united by only one surface. Thus, it will be noted that they were cases in which the grafts, because of free anterior and lateral surfaces, were able, to a considerable extent, to regenerate lost parts. With the regeneration of the parenchymatous tissue in the prepharyngeal region they were supplied with cells of their own species with which to replace those that became senescent, and thus they retained their specific characters. On the other hand, it is suggested that the situation, in the grafts which gradually developed host characters, was that the implanted tissues contained insufficient parenchyma cells to make the necessary replacements, and that this was accomplished by host cells.

Of considerable interest in this connection are the observations on page 230. It is well known (Stoppenbrink, 1905) that starvation causes a reduction first of the reproductive system and then of the parenchyma, digestive tract, and muscles, leaving the nervous system practically unaffected over a long period. In the case shown in Figures 53 and 54 it seems likely that starvation had reached a point where the implant was almost, if not completely, devoid of parenchymatous tissue. When feeding was reinstituted, it seems likely that host parenchyma migrated into the implant, for it soon began to take on the characteristics of the host species, although during the entire period of starvation the specific characters of the two components could be recognized. That host tissues do invade the graft is indicated by Steinmann's findings (1933). Using hosts vitally stained with *Cresylechtviolett*, he observed that the digestive tract of the host invaded the graft for a considerable distance.

GRAFT MIGRATION

In the experimental section was noted the migration of cephalic grafts in the pharyngeal region. When this migration is compared with regeneration of the controls, some striking similarities are seen. When a window is cut in a planarian, the surrounding tissues contract and a more posterior level is brought into contact with a more anterior one. During regeneration new tissue forms between the two levels until they are again separated by approximately the same distance as before. Thus, in the process of regeneration the more posterior level may be said to have "migrated" back to its original level. In terms of gradients, we may say that, when two gradient levels come in contact, new tissue forms between them until the lower level is again separated from the higher by cells representing the intervening levels of physiological activity.

It might appear, at first sight, that graft migration is fundamentally the same situation excepting that the difference in physiological level between the tissues in contact is greater. New tissue would be expected to form between graft and host until the intervening levels of the axial gradient are restored. This explanation does not, however, fit all the facts, for, according to it, the reconstituting pharynx would be expected to appear at the same level as the original one, that is, in the new tissue. This occurred in

the controls, but in the experimentals in every case but one the reconstituted pharynx was to be found caudad in the old host tissue.

The indications are that the experimental procedure in some way rejuvenates the piece which is grafted. If this is the case, it would explain the hypertrophy of the grafts and graft induced tissues in comparison to the uninfluenced regions of the host. It would also interpret the observation that on one occasion, when the laboratory temperature rose suddenly over a week-end, the grafts of a number of specimens disintegrated while the hosts remained intact. The greater muscular activity of grafts would also tend to indicate a higher general physiological activity, such as is characteristic of young planarians. If this should prove to be the case, then the facts observed regarding graft migration could be interpreted on the basis of a physiologically younger axis developing at the expense of the host tissues. Two processes are involved in a case like Figures 42 and 43: first, the development of a morphogenetic field behind the graft, and second, the elimination of tissue in front of the graft. That host tissues were actually destroyed is shown by the disappearance of structures on the left side of the host head as the graft approached, while the left side of the graft gave little evidence of suffering from its proximity to another dominant region.

If the graft is placed in anteroanterior orientation, it would be expected to migrate toward its level of origin; while if in anteroposterior orientation, it would migrate away from it. This is in agreement with observations of Santos (1931) on postcephalic grafts and with those reported here.

INDUCTION AND RANGE OF DOMINANCE

The very slight inductive ability of cephalic grafts in the head is in striking contrast to grafts in the pharyngeal and tail regions. Implants in the head induced outgrowths only when there was an exposed cut surface; and the supernumerary eyes which appeared when the grafts were ectopic or in reversed anteroposterior orientation are, at best, very questionable cases of induction. Since in two of the controls supernumerary eyes developed, there is a possibility that those of the experimentals were the result of regeneration by the host under slightly abnormal conditions. The occasional auricle-like structures may also have been regenerated rather than induced.

In the pharyngeal level, cephalic grafts have greater inductive power than in the head, though less than in the tail. Short to medium postcephalic outgrowths developed, and the level at which reconstituting pharynges appeared was considerably farther posterior than in controls. Since these developed by reorganization of tissue which had not been injured in the operations, and since no control showed such development, we may say that these pharynges were graft-induced.

Placed in the tail, cephalic grafts induced reorganization cephalad as well as caudad. Santos (1929, 1931) reported the induction of pharynges in reversed polarity by grafts in postcephalic regions but did not show the condition of the digestive tracts of these cases. Feeding the animals blood has made it possible, in many of the cases described here, to observe that the digestive tracts were completely reorganized between the graft and the induced pharynges both anterior and posterior to the implant. There were in certain cases structural evidences of induction at a still greater distance from the implant. In those a second tail developed between the original host pharynx and the more anterior graft-induced one (Fig. 83). Observations on the behavior of the compounds also indicated that graft dominance extended beyond the more anterior of the induced

pharynges. During locomotion there were well-defined zones under the influence of the host and of the graft. Stimulation of the graft resulted in contraction of the host as far anterior as the induced tail. On the other hand, stimulation of the host, unless severe, had little or no visible effect upon regions posterior to the induced tail.

Inductive capacity is associated with the ability to inhibit independent differentiation by tissues in the neighborhood of the inducer. The distance along the axis that this controlling action extends has been called "range of dominance" (Child, 1924). With the dominant region anterior, the controlling action extends in a posterior direction only. However, when a dominant region is placed in the pharyngeal region or tail, it may extend its control in an anterior direction as well. It is possible to obtain a rough measure of the anterior range of dominance of a graft by decapitation of the host at various distances anterior to the implant. Data from the decapitation experiments showed that the range of graft dominance was greater in the tail than in the pharyngeal region. In the case of grafts in the pharyngeal region "inhibitory dominance" seldom extended more than 2 mm. cephalad. On the other hand, grafts in the tail usually inhibited head regeneration from a cut surface as far anterior as the host pharynx, a distance of about 4–6 mm.

ROLE OF THE NERVOUS SYSTEM IN PLANARIAN INDUCTION

The fact that the heads of planarians are largely composed of nervous tissue would suggest the possibility that this tissue was responsible in some manner for the resistance of the cephalic level to induction. If this were the case, there should be a marked reduction in host resistance when implants were made directly behind the head in the anterior prepharyngeal region. Figures 31, 32, 33, 34, 35, 36, and 37 show that this was not the case. The results of Okada and Sugino (1934) on the Japanese *P. gonocephala* are of some interest in this connection. Their reported inductions of pharynges by implants from prepharyngeal regions show that in the Japanese species ganglionic material is not necessary for the reorganization of lower levels in the gradient. Santos (1931) did not report any inductions by implants from this level in *E. dorotocephala* and *E. tigrina;* but this matter probably should be reinvestigated, since it is known that pharynges will reconstitute in prepharyngeal pieces even if regeneration of a head is completely inhibited (Child, 1911*c*).

SUMMARY

1. A technique of operation involving rectangular transplants and ice as anesthetic gave a high percentage of takes between *E. dorotocephala* and *E. tigrina*. Four hundred and twelve cases of successful implantation of cephalic grafts were studied. Of these, 105 were in the head, 113 in the pharyngeal level, and 174 in the tail. Twenty were placed in the anterior prepharyngeal region for comparative purposes.

2. Union between the digestive tract of the implant and host was necessary for persistence and growth but not for regeneration of the graft. In all levels a free surface was necessary for regeneration by the transplant and determined its anteroposterior axis. Modifications of polarity up to 180° were observed. When union between graft and host was complete, there was no graft regeneration, and polarity apparently remained unmodified. Dorsoventrality of the grafts in all cases remained unchanged during the entire period of observation.

3. In most cases there was a gradual centripetal replacement of graft epithelia by

those of the host. There was some evidence that tissues other than the epithelia were also involved. When the amount of union between graft and host was slight, this replacement did not take place.

4. Cephalic grafts in the head commonly united with the hosts by all surfaces and became incorporated in them. Their epithelia and photoreceptors eventually were replaced by host tissue.

5. When the anteroposterior axis of hosts and grafts in the head region did not coincide, whether by accident or as a result of implantation in anteroposterior orientation, supernumerary photoreceptors occurred.

6. When grafts in the head did not unite completely, very short postcephalic outgrowths developed; and often from the free edge of the host window a more or less complete secondary head appeared.

7. In grafts in dorsoventral orientation there was more or less formation of new tissue between graft and host. In some cases this resulted in the development of additional heads, one surface of which developed from the implant and the other from the host.

8. Grafts in the nonganglionic, anterior prepharyngeal region showed very little more ability to induce than did those in the head, indicating that resistance to induction is not dependent upon the amount of nervous tissue present.

9. Grafts in the pharyngeal region induced larger postcephalic outgrowths than did those in the head. These were flat in cases of incomplete union but tubular in complete or nearly complete takes. A pharynx was induced posterior to the level of implantation in almost every case. In two cases a pharynx reversed in polarity was induced anterior to the graft.

10. A gradual migration was noted in a number of grafts in the pharyngeal region. This was most evident during inanition and was characterized by the appearance of new tissue behind the graft and the disappearance of host tissue anterior to it.

11. Cephalic implants in the tail region induced the largest outgrowths of tissue and the greatest reorganization of the host. This included reorganization of the two branches of the postpharyngeal digestive tract into a single prepharyngeal branch, development of pharynges anterior and posterior to the implant, and in a few cases a supernumerary tail between the pharynx of the host and that induced anterior to the graft.

12. Triangular cephalic grafts containing more anterior levels but less actual tissue than the rectangular implants showed greater inductive capacities than the latter.

13. Decapitation of the host anterior to the implants showed that the range of dominance of grafts in the pharyngeal region usually did not extend more than about 2 mm. cephalad. In the tail region, graft dominance usually extended about to the posterior tip of the host pharynx.

14. Double forms were developed from the posterior fission pieces of grafts with two tails. These possessed two pharynges, doubled prepharyngeal branches of the digestive tract, and more or less doubled postpharyngeal branches. Perpetuation of these by fission occurred, but they certainly could not be called hereditary modifications.

15. The gradient in host resistance to induction was strikingly illustrated in cases in which grafts were simultaneously implanted in different levels of the same host.

16. In the discussion the following topics are treated: (*a*) triangular versus rectangular grafts, (*b*) polarity, (*c*) replacement of graft by host tissue, (*d*) graft migration, (*e*) induction and range of dominance, and (*f*) role of the nervous system in planarian induction.

LITERATURE CITED

ABELOOS, M. 1930. Recherches expérimentales sur la croissance et la régénération chez les planaires. Bull. biol. France et Belgique, **64**:1–140.

BEYER, K. M., and CHILD, C. M. 1930. Reconstitution of lateral pieces of *Planaria dorotocephala* and *Planaria maculata*. Physiol. Zoöl., **3**:342–65.

BROWNE, E. N. 1909. The production of new hydranths in *Hydra* by the insertion of small grafts. Jour. Exper. Zoöl., **7**:1–24.

BURT, D. R. R. 1934. The capacity of different regions of *Pelmatohydra oligactis* Pall. to form head or foot. Jour. Exper. Zoöl., **68**:59–94.

CHILD, C. M. 1911*a*. Experimental control of morphogenesis in *Planaria*. Biol. Bull., **20**:309–31.

———. 1911*b*. Studies on the dynamics of morphogenesis and inheritance in experimental reproduction. I. The axial gradient in *Planaria dorotocephala* as a limiting factor in regulation. Jour. Exper. Zoöl., **10**:265–320.

———. 1911*c*. Studies, etc. III. Physiological dominance of anterior over posterior regions in the regulation of *Planaria dorotocephala*. *Ibid.*, **11**:187–220.

———. 1921. Studies, etc. XI. Physiological factors in the development of the planarian head. *Ibid.*, **33**:409–33.

———. 1924. Physiological foundations of behavior. New York: Henry Holt & Co.

———. 1927. Experimental localization of new axes in *Corymorpha* without obliteration of the original polarity. Biol. Bull., **53**:469–80.

———. 1929*a*. Physiological dominance and physiological isolation in development and reconstitution. Arch. f. Entw'mech., **117**:21–66.

———. 1929*b*. Lateral grafts and incisions as organizers in the hydroid, *Corymorpha*. Physiol. Zoöl., **2**:342–74.

———. 1931. Experimental modification of the scale of organization in the reconstitution of *Tubularia*. *Ibid.*, **4**:165–88.

———. 1932. Determination of new axes by lateral grafts and incisions in *Corymorpha* in relation to the gradient and dominance. *Ibid.*, **5**:593–627.

———. 1935. Dominance of hydranths induced by grafts in *Corymorpha*. Jour. Exper. Zoöl., **71**:375–87.

CURTIS, W. C., and SCHULTZE, L. M. 1934. Studies upon regeneration. I. The contrasting powers of regeneration in *Planaria* and *Procotyla*. Jour. Morphol., **55**:477–513.

FLEXNER, S. 1898. The regeneration of the nervous system of *Planaria torva* and the anatomy of double-headed forms. Jour. Morphol., **14**:337–46.

GEBHARDT, H. 1926. Untersuchungen über die Determination bei Planarienregeneraten. Arch. f. Entw'mech., **107**:684–726.

GOETSCH, W. 1926. "Organisatoren" bei regenerativen Prozessen. Die Naturwiss., **41**:1011–16.

———. 1929. Das Regenerationsmaterial und seine experimentelle Beeinflussung. (Versuch zur einheitlichen Beurteilung der regenerativen Erscheinungen.) Arch. f. Entw'mech., **117**:211–311.

HARRISON, R. G. 1921. On relations of symmetry in transplanted limbs. Jour. Exper. Zoöl., **32**:1–136.

———. 1936. Relations of symmetry in the developing embryo. Collecting Net, **11**:217–26.

HUXLEY, J. S. 1935. The field concept in biology. Trans. on Dynamics of Development, **10**:269–93.

HUXLEY, J. S., and DE BEER, G. R. 1934. The elements of experimental embryology. Cambridge University Press.

HYMAN, L. H. 1916. An analysis of the process of regeneration in certain microdrilous oligochaetes. Jour. Exper. Zoöl., **20**:99–163.

———. 1925. The reproductive system and others characters of *Planaria dorotocephala* Woodworth. Trans. Amer. Micr. Soc., **44**:51–89.

JANICHEN, E. 1897. Beiträge zur Kenntnis des Turbellarienauges. Zeitschr. f. wissen. Zool., **62**:250–88.

KEILLER, V. H. 1910. A histological study of regeneration in short head pieces of *Planaria simplicissima*. Arch. f. Entw'mech., **31**:131–44.

KENK, R. 1935. Studies on Virginia triclads. Jour. Elisha Mitchell Sci. Soc., **51**:79–125.

LANG, P. 1913. Experimentelle und histologische Studien an Turbellarien. I. Mitteilung. Heteromorphose und Polarität bei Planarien. Arch. f. mikr. Anat., **82**:256–70.

MILLER, F. S. 1937. Some effects of strychnine on reconstitution in *Euplanaria dorotocephala*. Physiol. Zoöl., **10**:276–98.

MILLER, J. A. 1937. Studies on heteroplastic transplantation in triclads. I. Cephalic grafts between *Euplanaria dorotocephala* and *E. tigrina*. Doctor's dissertation, University of Chicago Library.

MORETTI, G. 1911. Sulla trasposizione delle varie parti del corpo nella *Planaria torva* (Muller). Arch. ital. anat. embr. (Firenze), **10**:437–60.

MORGAN, L. V. 1906. Regeneration of grafted pieces of Planarians. Jour. Exper. Zoöl., **3**:269–94.

MORGAN, T. H. 1903. The control of heteromorphosis in *Planaria maculata*. Arch. f. Entw'mech., **17**: 683–95.

MURRAY, M. R. 1931. In vitro studies of planarian parenchyma. Arch. f. exper. Zellforsch., **11**:656–68.

MUTZ, E. 1930. Transplantationsversuche an *Hydra* mit besonderer Berücksichtigung der Induktion, Regionalität und Polarität. Arch. f. Entw'mech., **121**:210–71.

OKADA, Yô K., and SUGINO, H. 1934. Transplantation experiments in *Planaria gonocephala*. I. Proc. Imp. Acad., **10**:37–40.

PRZIBRAM, H. 1921. Die Bruch-Dreifachbildungen im Tierreiche. Arch. f. Entw'mech., **48**:205–444.

RAND, H. W.; BOVARD, J. F.; and MINNICH, D. E. 1926. Localization of formative agencies in *Hydra*. Proc. Nat. Acad. Sci., **12**:565–70.

RAND, H. W., and BROWNE, A. 1926. Inhibition of regeneration in planarians by grafting: technique of grafting. Proc. Nat. Acad. Sci., **12**:575–81.

RUSTIA, C. P. 1925. The control of biaxial development in the reconstitution of pieces of *Planaria*. Jour. Exper. Zoöl., **42**:111–42.

SANTOS, F. V. 1929. Studies on transplantation in *Planaria*. Biol. Bull., **27**:188–98.

———. 1931. Studies on transplantation in *Planaria*. Physiol. Zoöl., **4**:111–64.

SONNEBORN, T. M. 1930. Genetic studies on *Stenostomum incaudatum*. II. The effects of lead acetate on the hereditary condition. Jour. Exper. Zoöl., **57**:409–39.

STEINMANN, P. 1933. Transplantationsversuche mit vital gefärbten Tricladen. Rev. suisse de zool., **40**:269–72.

STOPPENBRINK, F. 1905. Der Einfluss herabgesetzter Ernährung auf den histologischen Bau der Süsswassertricladen. Zeitschr. f. wissen. Zool., **79**:496–547.

STRELIN, G. S., and TRIFONOVA, A. N. 1935. Fiziologicheskii gradient. Arch. Russes Anat. Hist. et Embryol., **14**:56–73.

WEISS, P. 1935. The so-called organizer and the problem of organization in amphibian development. Physiol. Rev., **15**:639–74.

WILLIER, B. H.; L. H. HYMAN; and S. A. RIFENBURG. 1925. A histochemical study of intracellular digestion in triclad flatworms. Jour. Morphol. and Physiol., **40**:299–340.

PLATE I

Photographs of Living Animals

Figs. 28–30, 33, 71, 83–86, 89–91, and 96.—*E. dorotocephala* into *E. tigrina novangliae*. Figs. 31 and 32.—*E. tigrina novangliae* into *E. dorotocephala*.

Fig. 1.—*E. dorotocephala*.

Fig. 2.—*E. tigrina*.

Fig. 3.—*E. tigrina novangliae* possessing mid-dorsal light stripe, type used in these experiments. Note pharynx (*P*).

Grafts in the Head Region

Fig. 28.—DVAA. Union complete. 273 days. Graft epithelia replaced by host tissue. Dorsoventral axis unaltered and no regeneration of host eyes. Same specimen shown in Fig. 27, 1 week after operation.

Fig. 29.—DVAA. Union by posterior part of dorsal surface only. 280 days. Graft (*G*) has regenerated head, tail, and functional pharynx. Species specificities kept during 10 months of observation.

Fig. 30.—DVAP. Posterior surface of graft failed to unite with host. 21 days. No host head developed during 133 days of observation.

Grafts in the Anterior Prepharyngeal Region

Fig. 31.—DDAA. Union complete. 175 days. Two auricle-like structures developed lateral to implanted eyes. Graft epithelia replaced by host tissues.

Fig. 32.—DDAA. Anterior surface of implant failed to unite with host. 180 days. Host tissue disintegrated to right of graft. Epithelia, head shape, and cephalic lobes of implant (*G*) typical of host rather than graft.

Grafts in the Tail

Fig. 71.—DDAA. Union complete. 252 days. Note tubular, graft-induced outgrowth with pharynx (*P*) posterior to, and with pharynx (*P'*) and very small projection anterior to, implantation level.

Fig. 83.—DDAA. Union by graft posterior surface only. 133 days. Reorganization of digestive tract with induction of pharynx (*P*) anterior as well as posterior to implant. Note tail (*T*) between host pharynx and anterior graft-induced one.

Fig. 84.—DVAA. Union by dorsal surfaces only. 21 days. Host fissioned during first week. Outgrowth of tissue does not involve ventral surface of host. Intestinal diverticula of graft failed to unite with those of host. Implant subsequently regressed and finally disintegrated.

Fig. 85.—DVAP. Union by posterior and left edge of graft. 28 days. Host window tore apart at posterior angles 1 week after operation, and sides have developed into tails. Unpigmented areas of three graft eyes (one *R* regenerated) seen through ventral surface.

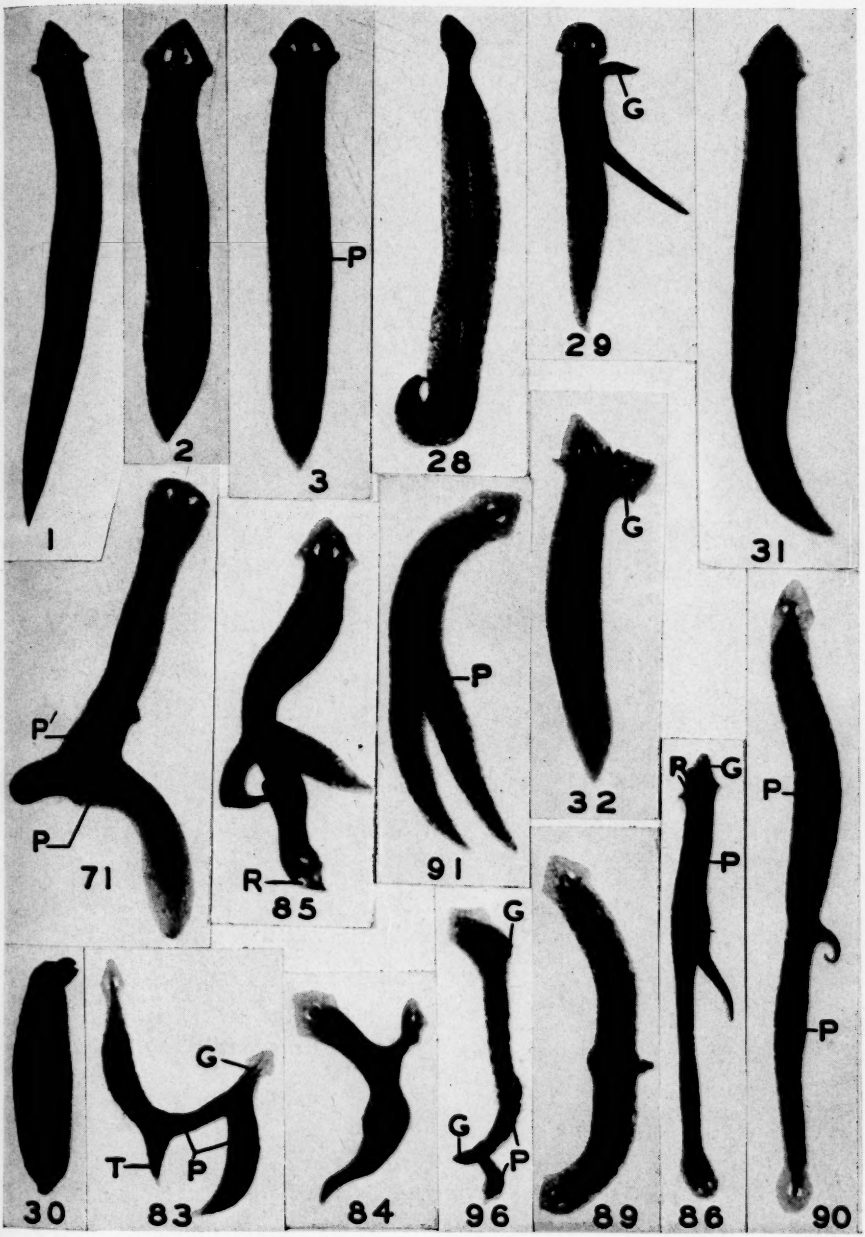
Fig. 86.—DVAP. Union complete. 70 days. Host fissioned immediately behind implant (*G*), and regenerated head (*R*) instead of tail opposite that of graft. Both share common ventral surfaces and pharynx (*P*) with reversed polarity.

Figs. 89 and 90.—DDAP. 56 and 70 days, respectively. Anterior surface of graft failed to unite with host. Window in host tore apart posterior to implant. Fig. 89.—Postcephalic outgrowth and pharynx with reversed polarity induced by graft. Small tail-like appendage developed from window. Graft epithelia replaced by host tissues. Fig. 90.—Photographed after feeding blood to show pharynges (*P*).

Fig. 91.—DDAP. Union by graft posterior surface. 154 days. Fission piece. Tail and pharynx (*P*) with reversed orientation developed in host anterior to implant. Fusion of tails along lateral surfaces in progress.

Fig. 96.—Two grafts (*G*). Both DDAA, union complete. 35 days. Teratophthalmic head regenerated on host after section 2 mm. anterior to first graft. Note extent of control of each head caudad and cephalad, and reorganization of digestive tract and induction of pharynx (*P*) anterior to second graft.

PLATE I



UNIV. OF MICH.